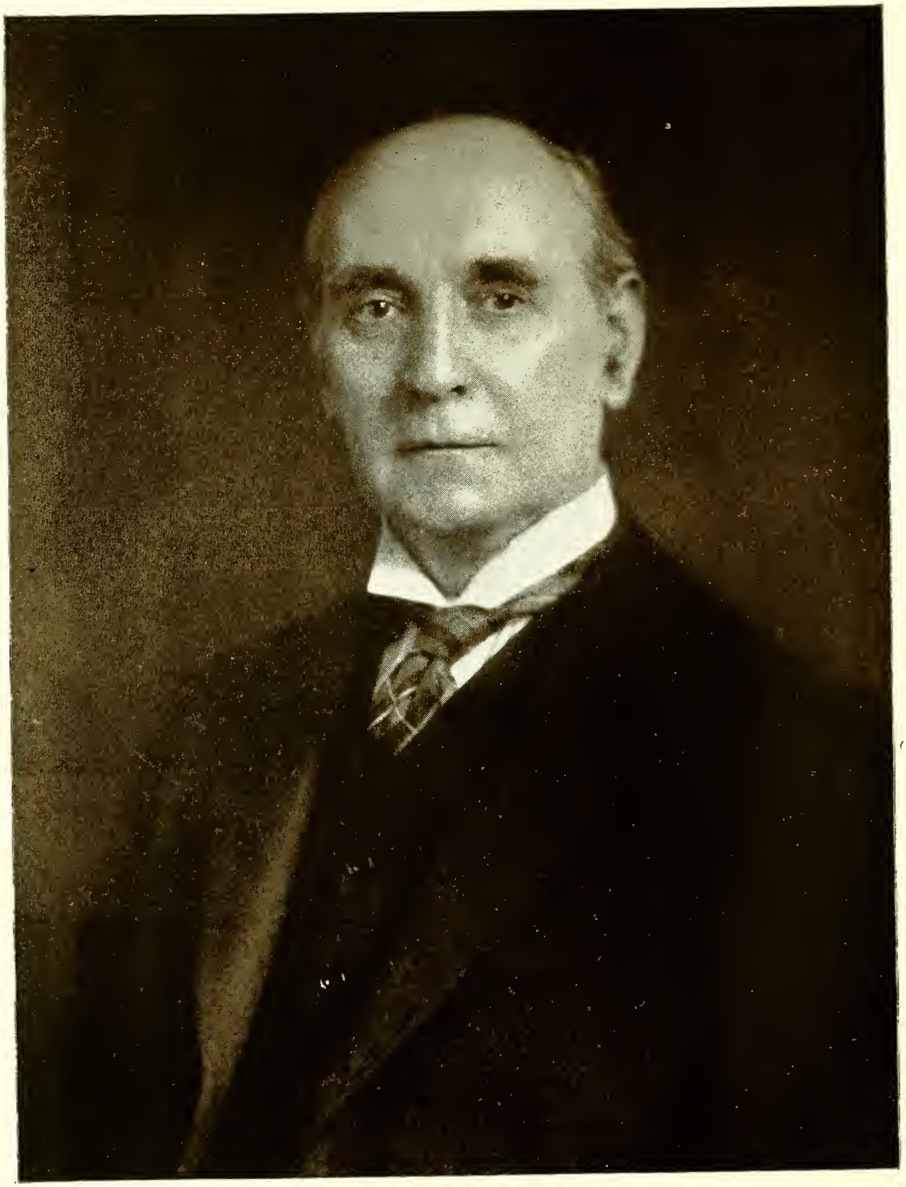

G. F. Stewart

Western Reserve University
Cleveland

Collected Papers

From

The H. K. Cushing Laboratory of Experimental Medicine

EDITED BY
J. M. ROGOFF, M.D.
ASSOCIATE PROFESSOR OF EXPERIMENTAL MEDICINE

Volume IX
1927–1931

TABLE OF CONTENTS

11. Studies on Adrenal Insufficiency. IV. The Influence of Intravenous Injections of Ringer's Solution upon the Survival Period in Adrenalectomized Dogs.
By J. M. Rogoff and G. N. Stewart.
(*Amer. Jour. Physiol.*, 1928, LXXXIV, 649–659.)

12. Studies on Adrenal Insufficiency. V. The Influence of Adrenal Extracts on the Survival Period of Adrenalectomized Dogs.
By J. M. Rogoff and G. N. Stewart.
(*Amer. Jour. Physiol.*, 1928, LXXXIV, 660–674.)

13. Studies on Adrenal Insufficiency. VI. The Influence of "Heat" on the Survival Period of Dogs after Adrenalectomy.
By J. M. Rogoff and G. N. Stewart.
(*Amer. Jour. Physiol.*, 1928, LXXXVI, 20–24.)

14. Studies on Adrenal Insufficiency. VII. Further Blood Studies (Cholesterol and Calcium) in Control Adrenalectomized Dogs.
By J. M. Rogoff and G. N. Stewart.
(*Amer. Jour. Physiol.*, 1928, LXXXVI, 25–31.)

15. Studies on Adrenal Insufficiency. VIII. The Survival Period of Untreated Adrenalectomized Cats.
By J. M. Rogoff and G. N. Stewart.
(*Amer. Jour. Physiol.*, 1929, LXXXVIII, 162–172.)

16. Studies on Adrenal Insufficiency. IX. The Influence of Extracts of Adrenal Cortex (Sheep and Cattle) on the Survival Period of Adrenalectomized Dogs and Cats.
By G. N. Stewart and J. M. Rogoff.
(*Amer. Jour. Physiol.*, 1929, XCI, 254–264.)

17. Blood Pressure Following Adrenalectomy.
By J. M. Rogoff and R. Dominguez.
(*Amer. Jour. Physiol.*, 1927, LXXXIII, 84–91.)

18. Functions of the Adrenal Glands.
By J. M. Rogoff and G. N. Stewart.
(*Amer. Jour. Physiol.*, 1929, XC, 497.)

19. Adrenalectomy and Muscle Fatigue.
By H. C. Stevens and J. M. Rogoff.
(*Amer. Jour. Physiol.*, 1930, XCIII, 691.)

20. On the Nervous Mechanism of Epinephrin Secretion.
By J. M. Rogoff, P. Wasserman and R. Hoecker.
(*Amer. Jour. Physiol.*, 1931, XCVII, 555.)

21. Pressor Effect of Guanidine Salts on the Non-anesthetized Rabbit.
By R. Dominguez.
(*Proc. Soc. Exp. Biol. Med.*, 1927, XXV, 57–60.)

22. The Systolic Blood Pressure of the Normal Rabbit Measured by a Slightly Modified Van Leersum Method. Final Report.
By R. Dominguez.
(*Jour. Exp. Med.*, 1927, XLVI, 443–461.)

23. Experimental Atherosclerosis and Blood Pressure in the Rabbit.
By R. Dominguez.
(*Jour. Exp. Med.*, 1927, XLVI, 463–485.)

24. Effect on the Blood Pressure of the Rabbit of Arteriosclerosis and Nephritis Caused by Uranium. Influence of Other Heavy Metals.
By R. Dominguez.
(*Arch. Path.*, 1928, V, 577–606.)

25. A Method of Blood Transfusion by Means of Rubber Tubes Vulcanized on the Blood Vessels.
By E. Karrer and J. M. Rogoff.
(*Amer. Jour. Physiol.*, 1929, XC, 406.)

26. Some Effects of Denervation on Muscular Contraction.
By H. C. Stevens and J. M. Rogoff.
(*Amer. Jour. Physiol.*, 1929, XC, 531.)

27. A Simple and Accurate Time Marker.
By E. Karrer and H. C. Stevens.
(*Science*, 1928, LXVIII, 87–88.)

28. A Head Clamp for Decerebrate Animals.
By E. Karrer and H. C. Stevens.
(*Jour. Lab. Clin. Med.*, 1928, XIV, 265.)

29. A Special Knife for Decerebration.
By E. Karrer and H. C. Stevens.
(*Jour. Lab. Clin. Med.*, 1928, XIV, 266.)

30. A Tendon Clamp for Measurements of Muscular Tension.
    By E. Karrer and H. C. Stevens.
    (*Jour. Lab. Clin. Med.,* 1928, XIV, 171.)

31. The Measurement of Muscular Tension and Its Bearing on Plurisegmental Innervation.
    By E. Karrer and H. C. Stevens.
    (*Amer. Jour. Physiol.,* 1928, LXXXV, 383.)

32. The Response of Negative After-Images to Passive Motion of the Eyeball and the Bearing of These Observations on the Visual Perception of Motion.
    By E. Karrer and H. C. Stevens.
    (*Amer. Jour. Physiol.,* 1930, XCIV, 611–614.)

33. The Measurement of Muscular Tension and Its Bearing on Pluri-Axonal Innervation.
    By H. C. Stevens and E. Karrer.
    (*Amer. Jour. Physiol.,* 1929, LXXXVIII, 686–697.)

34. Some Effects of Denervation on Muscular Contraction.
    By H. C. Stevens.
    (*Proc. Soc. Exp. Biol. Med.,* 1930, XXVII, 399–400.)

35. The Neurological Principles Underlying the Treatment of Muscular Atrophy.
    By H. C. Stevens.
    (*Ohio State Med. Jour.,* 1931, XXVII, 35–38.)

36. List of published papers, 1886–1930, by G. N. Stewart.

GEORGE NEIL STEWART. 1860–1930

University of Edinburgh

A.M.	1883
B.S.	1886
D.Sc.	1887
M.B., C.M.	1889
M.D.	1891
LL.D. .. Honoris Causa ..	1920

University of Cambridge

D.P.H.	1890

Demonstrator of Physiology, Owen's College, Manchester, England, 1887–1889.

George Henry Lewes Student, University of Cambridge, 1889–1893.

Examiner in Physiology, University of Aberdeen, 1891–1894.

Instructor in Physiology, Harvard Medical School, 1893–1894.

Professor of Physiology and Histology, Western Reserve University, 1894–1903.

Professor of Physiology, University of Chicago, 1903–1907.

Professor of Experimental Medicine, Western Reserve University, 1907–1930.

GEORGE NEIL STEWART

An Appreciation

By J. M. Rogoff

This volume includes the last collection of published papers representing work conducted in The H.K. Cushing Laboratory of Experimental Medicine under the Directorship of the late Professor George Neil Stewart. It also marks the end of a long period of intimate friendship and collaboration that existed between the great master, scientist, investigator and teacher, and myself. I take the privilege, therefore, of expressing a brief tribute to the memory of my eminent colleague and collaborator, to whom I am indebted not only for the happiness that was mine during the fifteen years of our joint labors but for much of whatever useful and creative service I may be privileged to contribute in the medical sciences.

Very few were so fortunate as to have had the rare opportunity of an intimate acquaintance with the personal qualities of Stewart especially in relation to his professional life and in the laboratory. Memories of the many attributes, which marked his superiority and genius, cannot but be cherished by his friends, as priceless possessions. They are spiritual gems which, in part, compensate for the personal loss sustained in being deprived of so great and inspiring an influence. It would be very pleasing to narrate many of the delightful incidents and experiences that occurred during my friendship with Dr. Stewart. However, impelled by my knowledge of his complete lack of vanity and his dislike for personal applause, I shall confine this appreciation to remarks concerning him as a teacher, investigator and collaborator.

As a teacher, Stewart brought to his pupils not only a thorough and mature knowledge of his subject but also the tremendous influence of a powerful personality. He made available the broadening influence of his enormous and ever enlarging store of information in fields other than physiology and he had a remarkable capacity for directing the student's mind toward independent quest for further knowledge and scientific truths.

An advocate and exponent of the laboratory and experimental method of teaching, he was responsible for valuable contributions to the promotion and teaching of physiology and other medical sciences. To him is due credit for the introduction of mammalian

experimental work for students, in the course of instruction in physiology, in the medical school. These principles, incorporated in his Manual and text-book of Physiology, exercised a tremendous influence in the development and progress of modern medical education not only in the United States but in the principal medical centers the world over. The pre-eminent rôle played by Stewart in establishing scientific teaching and investigation in Cleveland is reflected in the rapid development and growth of the Medical School, and in the policies and practises of other institutions. His influence upon medical education and investigation was favored by the high international standing gained through his publications and personal contacts. He actively participated in the promotion and support of the American Physiological Society and Journal and other societies and journals, both American and European, and his genius was in evidence during deliberations conducted at scientific societies and international congresses. In spite of his exceptional ability and great service as a teacher, the lure of the research laboratory was an ever-present factor guiding his principal interest in life. Consequently, when the opportunity came to devote himself entirely to experimental work he was ready to receive it. At the zenith of a most successful career as a teacher, enriched by years of experience and study, he dedicated the remainder of his mature life exclusively to experimental investigation.

Stewart's life in the laboratory, his relations to the work conducted therein and the contacts with his associates were characterized by a remarkable individuality. This created a thoroughly stimulating atmosphere. Administrative duties were relegated to a minor place in the necessary activities. Performance of the primary functions of the laboratory alone determined administrative policies. In scientific investigations, Stewart was a most thorough and untiring worker. He attacked problems with tenacity; an admirable persistence afforded him aid in conquering many technical difficulties. In his laboratory was found a welcome to anyone who could demonstrate ability, industry and honesty of purpose. These were the only requirements.

Stewart's well-known mastery in the literary art rendered his written and verbal communications unique. These were characterized by superb diction, a capacity for the use of delightful trope and metaphor and often a subtle manifestation of his natural endowment of a keen sense of humor. These attributes enabled him to

cast oil upon turbulent waters in the oft-encountered storms of present day scientific discussions. Nevertheless, he was not given to concealing an antipathy toward uncritical scientific work or reasoning. He was a vigorous exponent of the principle that the result of an investigation is no better than the method and skill with which it is conducted.

Only the highest attainable standards satisfied Stewart in scientific work or in the logic and reasoning applied to it. This characterises all of the contributions made by him. He explored and enlarged all the major avenues in the fundamental medical sciences. His earlier studies were in the field of bio-physics or electrophysiology, to which he made a number of notable contributions, especially in the physiology of nerve and muscle. Stewart was the first to make studies, by a suitable graphic method, on the action of the vagus and sympathetic nerves of the heart, in relation to temperature and endocardiac pressure. Later, his attention was directed to work in the physiology of the blood and its circulation, which led to his outstanding contributions on hemolysis and on circulation time. He made studies on the spleen, heart, intestine, lung, etc., comparing measurements of the circulation time observed by the dye method with those obtained by the electrical method. His early studies on cell permeability and on hemolysis led to a better understanding of the effects of hemolytic agents. Stewart made very valuable observations on the relation of hemolysis to the electrical conductivity of the blood. His extensive studies on the conductivity of the blood and its components led to his development of an exceedingly accurate electrical method for estimating the relative volume of corpuscles and plasma.

During the four-year interval, from the time he relinquished the chair in physiology at Western Reserve University to succeed Jacques Loeb in Chicago, until his return to Western Reserve University to become Director of The H.K. Cushing Laboratory of Experimental Medicine, he conducted extensive studies on resuscitation and on acute cerebral anemia, in collaboration with C. C. Guthrie and F. H. Pike. On his return to Cleveland, he developed his well-known calorimetric method for measuring the rate of bloodflow in the extremities, and devoted a number of years to valuable investigations on the circulation of the blood in the extremities, in health and in certain diseased conditions. During the last fifteen

years of his life his scientific interest was largely devoted to our experimental studies in Endocrinology.

Stewart's Manual of Physiology was recognized as one of the leading text-books on this subject in the English language. It was written in the characteristic style of the author. The first edition was published in 1895. Having undergone eight revisions, the last edition appeared in 1918. Numerous requests were made by colleagues for another edition of his Manual, but he was prevented from undertaking the laborious task by a prolonged state of ill health which followed a severe attack of influenza in 1923. In spite of this handicap he maintained his interest in the laboratory up to the time of his death. His last contribution was an extension of studies begun forty years previously. This paper, dedicated to E. Gley and J. F. Heymans, heads the list of collected papers in this volume. A complete list of his scientific publications has been included in the volume, the references being arranged in chronological order. These contributions bear monumental witness of Stewart's great service to science and to humanity.

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VOLUME XXXVIII

DÉDIÉ A E. GLEY ET J. F. HEYMANS

EXTRAIT

MEASUREMENT OF THE TEMPERATURE OF THE SKIN

G. N. STEWART

GAND
RÉDACTION DES ARCHIVES
3, QUAI BAERTSOEN

PARIS
O. DOIN, ÉDITEUR
8, PLACE DE L'ODÉON

1930

FROM THE H. K. CUSHING LABORATORY OF EXPERIMENTAL MEDICINE,
WESTERN RESERVE UNIVERSITY, CLEVELAND, U. S. A.

MEASUREMENT OF THE TEMPERATURE OF THE SKIN

G. N. STEWART

Forty years ago I published a paper on this subject (1). The observations were made in connection with an investigation of the loss of heat by radiation from the body. The data are similar to those communicated in recent years by a number of writers, especially BENEDICT and his pupils (2), (3), none of whom refer to my work. They employed thermocouples, which were used nearly a century ago for the same purpose by BECQUEREL and BRESCHET, and later on by others. The work of KUNKEL (4) by this method was particularly good. BENEDICT's apparatus is of course an advance on any thermoelectric arrangement hitherto used for skin temperature measurements, but possibly he attributes undue importance to this particular method. There are several ways in which the skin temperature can be measured, but the chief difficulty is to know what to do with the data once we have got them.

In my previous research I employed « electrical resistance thermometers of lead paper cut out into a grating (fig. 1). The resistance was between three and four ohms. The resistance thermometer applied ot the skin was balanced in the Wheatstone bridge by another of precisely similar construction and nearly of the same resistance and the bridge completed by a graduated wire with sliding contact. » No box was required in the bridge, but an appropriate resistance was introduced into the battery circuit to regulate the current so that the galvanometer deflection should be sufficient to yield the required degree of accuracy. The current was kept weak and « creeping » of the deflection was not present. While, as remarked by A. V. HILL, the resistance method

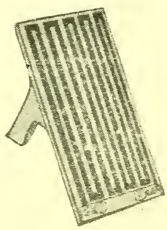

FIG. 1. — Resistance thermometer (lead paper). Natural size.

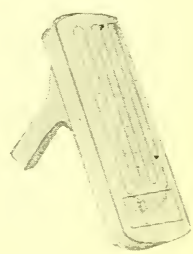

FIG. 2. — Resistance thermometer (platinum). Natural size.

is not usable where high sensitiveness is required, as in his experiments on heat production in nerve, it gives good results in the measurement of skin temperatures. A few of the data obtained in the previous research may be reproduced here (tables 1 and 2) as they seem to have passed completely unnoticed by subsequent writers, although agreeing closely with data published in quite recent years secured by thermo-electric methods. My work on this subject was referred

TABLE 1

Deflection read { Δ = difference of temperature between the part and the air of the toom. T = temperature of the part.

Region	Deflection	Δ	T	Temperature of room
Anterior surface of left forearm	222	16.8	34.4	17.6
Posterior surface of left forearm	218	16.4	34.0	17.6
Anterior surface of left arm over belly of biceps	232	17.6	35.0	17.4
Left leg over head of tibia	190	14.4	31.9	17.5
Skin just below xiphoid cartilage	226	17.2	34.7	17.5
Skin over sternum	206	15.6	33.2	17.6
Trousers over anterior surface of left thigh (nothing between trousers and skin)	82	6.1	23.7	17.6

TABLE 2

Position of the slider read.

Region	Δ	T	Temperature of room
Palm of left hand	12.58	30.95	18.37
Forehead	14.70	33.04	18.34
Right cheek	14.92	33.21	18.29
Left breast	16.01	34.40	18.39
Right hypogastrium	17.00	35.16	18.16
Over apex beat	16.32	34.57	18.25
Sole of left foot	12.75	31.05	18.30

to in the first edition of my textbook of Physiology (5) published in 1896, and in all subsequent editions.

Several forms of grating were employed. It was thought that covering an area of skin of a certain size with a resistance thermometer might tend to increase its temperature beyond the normal. To avoid this

objection gratings were « designed to allow part of the area whose temperature was being measured to be still in free communication with the air, or perhaps more correctly, to measure the average temperature of alternate strips of the given area. A lead paper grating was cut out after being attached to a nonconducting base and the material of the base was then out away in the intervals between the bars of the grating. The breadth of an interval was the same as that of a bar. »

Another form of grating was devised « to allow the outer surface of the bars of the grating to be expossd to the air. » Observations made with the three forms of resistance thermometer described did not differ greatly from each other. Naturally the conditions were kept as uniform as possible in the different observations, especially the room temperature.

In connection with the observations on radiation of heat from the skin, including the outer surface of the clothes (normally the most important of the radiating surfaces), which constituted the chief subject of the paper, simultaneous measurements of the surface temperature and the radiation were made in a number of experiments. These yielded results of considerable interest but need not be quoted here as the present paper is only concerned with skin temperature. One curious point may, however, be referred to, namely that the skin has a remarkably high coefficient of emission. I found that covering a portion of the palm with a thin layer of lampblack caused little change in the rate of radiation. I have not seen this observation referred to by writers who many years later suggested that as regards radiation the skin behaves like a black body.

In the matter of the practical measurement of skin temperature I drew the conclusion that almost any form of resistance thermometer (and of course also of thermo-junctions) would be a great improvement upon the mercury thermometer as used up to the time I wrote, whether flat-bulbed or spiral-bulbed or the ordinary straight thermometer. With all of these the common procedure was to apply the thermometer to the skin without effectively preventing loss of heat from the uncovered surface of the bulb. The results were bound to be inaccurate and, working with the straight thermometer, I easily convinced myself that the procedure was useless.

But, it is easy to arrange a clinical thermometer to read skin temperatures accurately. All that is necessary is to insert the bulb into a cylindrical opening bored in the narrow edge of a small wedge of cork (fig. 3). The cork should be of good quality and the sharp edge should be smoothed with sand paper. The wedge can be made

with a sharp knife and a small drill in a few minutes and will last indefinitely. The bore is run so as just to open the edge of the cork thus permitting a narrow strip of the bulb (generally a little over 1 mm.) to come into contact with the skin when the thermometer is held in position with the sharp edge on the skin. Generally not more than a strip of the cork about 2,5 mm. wide is in contact with the skin. The bore is of such a calibre that the bulb when pushed into it is firmly grasped. It is therefore everywhere in close contact with the cork (Fig. 4). When in position on the skin the bulb is completely protected against exposure to the air and therefore quickly assumes the temperature of the skin. Naturally, a sufficient time must be allowed for the thermometer to register its maximum. With the so-called « one-minute » thermometer I generally allowed 2 or 3 minutes. An erroneous result will necessarily be obtained if an arbitrary time of contact is taken. That is the case with resistance thermometers or thermo-junction also. The true skin temperature is indicated when the thermometer has ceased to rise. A more prolonged contact than that mentioned is not under ordinary conditions required. It need scarcely be said that the pressure with which the bulb is held against the skin should be as uniform as possible. It is not necessary to indent the skin in any marked degree. The slightly yielding cork wedge acts as a cushion to prevent this. The fingers which grasp the stem of the thermometer easily learn to apply a uniform gentle pressure. Theoretical objections can be ruled out, for example that the thermometer will cool the skin and so alter its temperature. It is enough to point out that, as in measuring the temperature of the cavities the thermometer can easily be brought to a temperature a degree or two below that expected before application to the skin. In any case the heat capacity of the small bulb is not great. It is seldom that very rapid fluctuations of temperature occur on a skin area unless vasomotor changes are purposely induced. To detect the beginning of such rapid changes as the preliminary reflex vasoconstriction caused by application of heat even the smallest mercury thermometer will have too much lag, although

Fig. 3. — Cork wedge for clinical thermometer. Natural size.

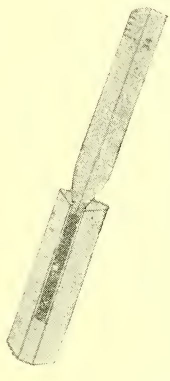

Fig. 4. — Clinical thermometer with the cork wedge attached. Natural size.

the vasoconstriction can be very well made out by the large thermometers which I employed in my calorimetric method of measuring the blood flow in the extremities. The normal result of plunging one hand into warm water (not hot enough to cause pain) is to diminish the rate of heat loss to the calorimeter by the other hand. This reflex vasoconstriction is succeeded by a more prolonged reflex vasodilatation. I published an account of this reaction with numerical data in my first paper (6) on the measurement of blood flow in the extremities and in many succeeding papers. It was also described in my text-book (5) in the 7th edition (1914) and in subsequent editions. Much later the vasoconstrictor reaction caused by heat was studied by MARTIN and JACOBY (7), who confirmed my results, without, however, mentioning them. They employed an electrical resistance thermometer and do notseem to know that this method was used by me more than 30 years before their work. I have shown by means of the electrical resistance thermometer that the reflex vaso-constriction begins a fraction of a second after the immersion of the contra-lateral hand in warm water.

Formal discussion of suggested objections to the use of the mercury thermometer in the way described is seen to be superfluous when the readings on one and the same area of skin are compared with those obtained with a thermometer of the other type. The two thermometers are applied simultaneously or successively. The results are identical, or at most only such small differences are found as exist in successive readings with one and the same thermometer. It is unnecessary to say that all the mercury thermometers are corrected by comparison with a standard thermometer. An abridged protocol of a typical experiment follows. The newer resistance thermometers were constructed either of platinum wire, 0.1 mm. in diameter, or of lead paper. The metal was fastened by a thin layer of thick varnish on a bakelite base. In putting on the platinum wire small pins were inserted at the turns but withdrawn when the varnish had set. Thereafter nothing more than the varnish was necessary to keep the wire in position. The distance between the skin and the wire is very small but the elecrtical insulation is excellent. The ends of the wire (or lead paper) are connected with small brass binding posts the heads of which are counter sunk in the bakelite so that they cannot touch the skin. In addition the binding posts are covered with a good coat of asphaltum. The resistance thermometer which is to be placed on the skin is provided with a small wooden handle at the back by means of which contact of the face of the thermometer with the skin is ensured, the handle being held by the observer so that the thermometer is completely in contact with the skin area. Figs. 1 and 2 show the thermometers

in the natural size. Figs. 3 and 4 show the mercury thermometer with its cork wedge in the natural size.

Mar. 5. 2:05 p.m. Room temperature 25.1°. The weather is cooler than for some days. Outside temperature 2.2° to 4.0° C. (dry bulb); humidity 54 to 59. Room feels rather cool and hands cooler than for some days. Both resistance thermometers at same temperature (19.05°). Slide wire reading 528 : 472. This gives the permanent ratio of the resistances of this pair of thermometers. The resistances were 4.48 and 4.00 ohms. It would have been easy to make the resistances more nearly equal, but as the results were quite satisfactory there seemed no point in troubling about this. 2:11 p.m. Handle thermometer (that is the one provided with a handle for application to the skin) placed in warm bath. 2:23 p.m. Battery and galvanometer circuits closed. Deflection 249, reaching maximum in about 30 seconds. 2:25 p.m. Bridge balanced to bring deflection back to zero. Reading of slide wire 535.0. Temperature of warm bath 35.27°, of cool bath (in which the balancing resistance thermometer still lies) 19.12°. 2:28 p.m. Both thermometers still in the baths. Battery and galvanometer again closed. Deflection 252 in 30 seconds. Temperature of warm bath 35.26°. For the first observation 1° difference between the baths corresponds to 15.4 divisions deflection; for the second observation it is 15.6 divisions. 2:58 p.m. Handle thermometer returned to warm bath. 3:06 p.m. cool bath 19.37°, warm bath 33.89°. 3:12 p.m. Deflection 222 or 15.3 divisions per degree difference of temperature of the two thermometers. 3:15 p.m. Handle thermometer removed from warm bath and at 3:18 p.m. placed on palm of left hand (ulnar side, one cm. below wrist). Deflection 232 in 2 minutes, 5 seconds, (the time required to reach maximum), moving first rapidly then slowly. A good steady maximum. Cool bath 19.41°. Temperature of area on palm 34.57° by resistance thermometer.

3:22 p.m. to 3:28 p.m. Clinical thermometer placed on same area of palm twice. Temperature of palm 34.64°. 3:38 p.m. Handle thermometer placed on same area of palm. Deflection (in 3 minutes) 225: cool bath 19.50°. Skin temperature 34.2°. 4:05 p.m. Handle thermometer again placed on palm. 4:08 p.m. deflection 222. Cool bath 19.65°. Temperature of palmar area by resistance thermometer 34.16°. The clinical thermometer gave 34.10°. Mouth temperature 37.1°. Air temperature 24.5°.

A number of other experiments gave similar results. It will be seen that the agreement between the temperature readings by the resistance and mercury thermometer is excellent. If in some of the experiments a difference of as much as 0.5° was occasionally seen this could sometimes be traced to insufficient duration of contact of one or the other thermometer. It was not found advisable to attempt to adjust the pressure in any other way than by estimating it with the fingers which held the thermometers. But in all cases it was light, and with light pressure it is perfectly feasible to maintain complete contact for the necessary time.

In table 3 are given a series of readings with the clinical thermometer taken on the same area of the palm from time to time over a period of 10 months. In some cases the temperature of an area on the dorsum of the hand was taken about the same time usually on the skin between the metacarpals of the thumb and index finger. Where the term

« dorsum » is employed without specification this area is always meant. The area on the palm was always the same, a strip on the ulnar side of the left palm extending downwards from a level about 15 mm.

TABLE 3

Date	Hour	Temperature			
		Palm	Dorsum	Mouth	Room
May 4	1:00 p.m.	35.56			27.1
	1:35 p.m.	35.70		37.1	26.7
	4:00 p.m.	35.59			26.7
May 5	10:40 a.m.	35.70		37.2	27.9
May 6	12:00 m.	35.74		37.2	28.4
May 14	11:20 a.m.	36.30		37.4	29.9
May 16	7:30 p.m.	36.34		37.15	28.3
May 17	9:05 a.m.	35.95		37.1	26.3
May 19	12:15 p.m.	35.14		37.0	24.3
May 20	11:00 a.m.	35.80		37.1	26.7
	4:00 p.m.	35.52		36.92	25.8
May 21	9:45 a.m.	35.75		36.93	26.3
May 22	9:30 a.m.	35.90	34.12	37.23	26.6
May 23	1:30 p.m.	35.93	35.36	37.1	27.9*
May 26	10:30 a.m.	35.88	35.22	37.24	25.85*
May 27		36.5	34.90		28.3
June 2	1:30 p.m.	35.37	34.0	37.20	25.6
June 6		36.08			25.6
June 8	7:00 p.m.	36.20	34.56†	37.10	27.2*
Feb. 11	3:30 p.m.	35.88	34.0	37.18	27.71
Mar. 5		34.64		37.1	25.2
Feb. 24	2:19 p.m.		34.0		
	2:25 p.m.		34.0		26.18
	4:05 p.m.		34.48		26.6

* The temperature of an area on the brow was measured on these dates at about the hours mentioned. It was a horizontal strip between the eyes 35 mm. above the level of the inner canthus. Temperatures were 35.38°, 35.45° and 34.53° respectively.

† In this observation the temperature was taken on the dorsum of the proximal phalanx of the middle finger.

distal to the wrist. The hour of the day at which each temperature measurement was made is generally noted. This gives approximately the interval since the last meal, for the subject had breakfast regularly about 8 to 8:30 a.m., dinner at 5:30 to 6 p.m. and no lunch. He was in good health with the exception that both feet and the palms of the hands were hypo-aesthetic, following an attack of influenza. The dorsum of the hands was normal and their motility was unimpaired. Clinically the condition appears to have been stationary for at least

a year at the time the temperature observations were made. In a warm atmosphere the palms tended to become red. Sweat secretion seemed unimpaired. It is probable that the temperature of the palm areas was always increased somewhat by the relatively high temperature of the room and of course by the resulting vasodilatation. The results on the palm are therefore not to be considered entirely normal. But that does not hinder them from being compared with each other, and the uniformity shown in table 3 over so long a period as 10 months is remarkable. It has already been stated that the clinical condition remained stationary, and therefore it might be expected that the skin temperature reaction to an external temperature which also changed but little should not vary much. This might be taken as indicating the utility of skin temperature measurements in judging the clinical condition in these and similar cases. In considering uses for such measurements, however, it is necessary to remember that variations due to changes in the environment, especially in the air temperature, must be sedulously controlled.

The temperature of the areas on the dorsum of the hand, both the area habitually chosen and other areas occasionally used, may be considered as normal for the room temperature. That they are somewhat higher than corresponding skin temperatures observed in my earlier experiments (tables 1 and 2) is fully explained by the great differenec between the cool, often uncomfortably cold, British laboratories and the over-heated American ones. The uniformity of the skin temperature readings throughout the 10 months is about the same on the normal areas of the dorsum as on the somewhat hyperemic palmar areas. The readings from the dorsum of this hand did not differ materially from readings taken on corresponding areas of two healthy young men.

A reference to the protocol of June 14 containing observations on the left hand of M. (dorsum between metacarpals of thumb and index finger) illustrates this. M. was a healthy young man of heavy build. His hands were much larger than those of S. The clinical thermometer gave a temperature of 35.0° on the dorsum of the left hand in the usual position between the metacarpals of the thumb and index. With the platinum resistance thermometer in the same area the skin temperature was 34.75°. Room temperature 26.67°.

The subacute pathological condition on the palm of S. was not considered a disadvantage for the temperature measurements else a normal for the temperature measurements else a normal palm could have been chosen. The hypoaesthetic and usually somewhat hyperemic palm was purposely chosen because the condition was so definitely stationary.

Some new observations were made with the lead paper resistance thermometer. One was placed on dorsum of a normal hand (M) at the usual position. The temperature of the skin was calculated at 34.85°. Deflection 195, and 26 divisions = 1°. With the clinical thermometer it was 34.80°. Room temperature 26.85°. The resistances of the lead paper thermometers were not much different from those used in the previous work (3 to 4 ohms). The deflection began less than 1 second after application of the thermometer to the hand. These thermometers were very quick in following the temperature of the skin. The lead paper was fixed with the thick varnish in the same way as the platinum wire and nothing except the varnish was interposed between the metallic grating and the skin. The platinum thermometers were possibly somewhat easier to make than the lead and were used much more in the later observations.

Some readings were made of the temperature of the skin after immersion of the hand in a bath of known temperature. The bath was so large that in 10 minutes the heat given off by a hand caused only a small change in its temperature. The object was to determine the temperature readings obtained on the surface of the part after immersion in the preliminary bath, according to the calorimetric method of measuring blood flow in the extremities. The temperature of the bath was a few degrees below that of the arterial blood coming to the hand. In previous observations (6) this was shown to be 0.5° below the rectal temperature in a healthy man (C.) with arms lightly clothed and seated in a comfortably warm room. The determination was made by starting with a temperature of the preliminary bath and calorimeter a little above blood temperature. When the hand was placed in the calorimeter the calorimeter temperature fell for a time because the venous blood left the hand at a temperature above that of the arterial blood. Finally the calorimeter ceased either to lose or gain heat from the blood. At this point the calorimeter temperature must have been the same as that of the arterial blood entering the part.

In the observations on the effect of immersion of the hand in a large bath on the surface temperature of a given area, the hand on withdrawal from the bath was rapidly dried by porous papar and a smooth towel without rubbing and the thermometer (mercury or resistance) at once applied.

Thus in the experiment of June 4 the left hand (at 6:30 p.m., one hour after dinner) of S. was left 10 minutes in a bath whose temperature was 32.82° when the hand was taken out. The clinical thermometer applied to the dorsum (knuckle of middle finger) for 3 minutes showed 33.20°. Room temperature 29.4°. Two hours later the same skin area showed a temperature of 34.87°. Room 27.3°.

Experiment of Feb. 12, 3:30 p.m. Left hand immersed in large bath (at 30.07°) for

10 minutes Drying occupied 25 seconds. At 3:41 p.m. the handle thermomete- was placed on the dorsum of the first phalanx of middle finger. It showed a skin temperature of 31.11°; about 1° above the temperature of the bath.

In another experiment (Feb. 20) the hand was placed for 10 minutes in a bath of temperature 29.40°, which had fallen to 29.31° when the hand was taken out. Temperature of the skin on dorsum of first phalanx of middle finger by resistance thermometer was 29.55°, only slightly higher than the bath.

Other experiments gave similar results. It is not necessary to enquire what are the components of the « skin temperature » measured after the preliminary bath. They are the same as those which enter into the reading before the bath, that is to say the temperature of the medium in contact with the skin; especially the rate at which heat is given off to it by conduction,convection and radiation; and the rate at which this heat is being replaced especially by the circulation and by conduction from deeper layers. It is impossible to give a definite value to the thickness of the layer of tissue which contributes to the skin temperature reading. The bearing of the observations on the establishment of « steady » temperatures in the part whose blood flow is being determined is that they enable us to follow to some extent the modification of the hand temperature produced by the preliminary bath. The object of the bath is to prepare the hand for the calorimeter by allowing the changes in the temperature of the hand at different levels produced by immersion in water at a temperature a few degrees below that of the blood to take place, at least approximately, before the hand is transferred to the calorimeter. If the temperature of the different levels of the hand now remains nearly the same during the time (generally relatively short) of immersion in the calorimeter, the heat given out to the calorimeter must have come from the blood, not in any important amount from the store of heat in the tissues of the hand. It is easy to show that any possible fall of average temperature of the hand in the calorimeter after the preliminary bath could cause only a negligible effect in comparison with the heat brought to the hand by the blood.

The technical problem of measuring the so-called skin temperature is sufficiently well solved and the clinician has the choice of several methods. I recommend the clinical mercury thermometer as being the simplest instrument and one with which all clinicians are familiar. I mention this merely because clinical men keep writing me for advice as to using thermo-junctions. They certainly can be used and will give good results but most clinicians will find it easier to employ the mercury thermometer. I do not advise clinical men to measure skin temperatures as a routine. Only rarely will information of value be obtained in this way. Differences of temperature at different areas of the exposed skin have long been known to exist and have often

been measured. Under ordinary conditions in temperate climates only the face, hands and sometimes the feet are exposed. The temperature at different parts of the clothed skin has litle physiological significance. The tendency of the clothing is to obliterate the differences which may be detected when the body is exposed. Whether some differences can still exist is a matter of little importance because this depends almost entirely upon the amount and nature of the clothing. With thick clothing covering everything except the hands and face the differences almost or altogether disappear.

No doubt under certain pathological conditions differences of skin temperature may be accentuated. Important observations in these conditions were made long before the introduction of clinical thermometry.

SUMMARY

This work is in continuation of observations published by the author forty years ago, « On the conditions which affect the loss of heat by radiation from the animal body. » In that research it was necessary to measure the temperature of the skin at various points under definite conditions, especially the temperature of the air. Electrical resistance thermometers of lead paper were employed and yielded satisfactory results. The heat capacity of the thermometers was very small, and their adjustment to the temperature of the skin was rapid. The galvanometer deflection quickly reached a maximum. The current was, of course, kept as weak as was consistent with a sufficient deflection.

I have since extended the work in certain directions, especially in comparing the results obtained from clinical thermometers fitted with a wedge of cork to prevent loss of heat to the air with those yielded by resistance thermometers (generally of platinum wire). The agreement in the temperature readings of the two thermometers on a given skin area was extremely close. For the clinical worker who desires to measure skin temperatures the ordinary maximum thermometer with a small bulb used in the way described is to be recommended.

Readings were taken on the hand of the same individual over a period of 10 months, covering summer and winter. The mercury thermometer was usually employed but electrical resistance thermometers were also used to check the results. The great majority of the observations were on two areas of the hand, one on the dorsum, the other on the palm. The readings for the same area throughout the 10 months were surprisingly constant. The main reason for this was the almost uniform room temperature. Also the routine of the

subject, especially as regards meals, was extremely regular. Further, his clinical condition remained unaltered throughout the period.

The reflex vasoconstriction caused by immersion of a hand in warm water observed by me in work on the measurement of the blood flow in the extremities by the calorimetric method (8) was further studied by the resistance thermometers. When one hand was placed in warm water the preliminary vasoconstriction began in the other in a fraction of a second. As was shown in the calorimetric observations the vasoconstriction is followed by a vasodilatation. This is very easily followed by the resistance thermometer.

Some observations on the temperature of the hand areas were made after immersion of the hand in a bath of known temperature for 10 minutes. These have a bearing on the use of the « preliminary bath !» in the calorimetric method of measuring blood flow. The readings of the mercury thermometer and the resistance thermometer (platinum) showed good agreement.

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On the Conditions which affect the Loss of Heat by Radiation from the Animal Body

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On the Conditions which affect the Loss of Heat by Radiation from the Animal Body.*

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THE loss of heat by radiation per unit of surface of a body in an enclosure of constant temperature depends upon two factors, (1) the nature of the surface, and (2) the excess of temperature of the surface over that of the enclosure. It is only, however, in vacuo that a body is ever cooled entirely by radiation. When the enclosure contains gas there is always surface conduction. When the excess of temperature is small, the loss of heat varies approximately as the product of this excess into a constant, the coefficient of emission or emissive power, which depends upon the nature of the radiating surface. In this, the simplest, case, we have

$$-\frac{d\theta}{dt}=c\theta \qquad (1)$$

as the expression for the rate of cooling, where θ is the excess of temperature, and c a constant.

Dulong and Petit have given the following formula, which agrees fairly well with the observed facts for a wide range of temperature:

$$-\frac{d\theta}{dt}=c\left(a^{\tau+\theta}-a^{\tau}\right) \qquad (2)$$

where θ is as before the excess of temperature of the radiating body, τ the temperature of the enclosure, c a constant depending on the nature of the body, and a an absolute constant which for the centigrade scale is 1·0077. Various other formulæ have

* This paper is intended to sketch an experimental method and to outline a discussion rather than to give a sustained and detailed account of results. A few of the measurements have been given, however, as illustrations.

[The work was begun in the Physical Laboratory at Edinburgh in the Summer of 1885.]

been deduced. In all of them the two factors mentioned above are of course involved; and it will be sufficient for our purpose to observe that for such differences of temperature as exist under normal circumstances between the skin and the air of a room, the rate of cooling of a thermometer or a small metallic ball is somewhat greater than that given by formula (1)—for a difference of temperature of 20°, not more than 6·5 per cent greater.

When we come to the problem of radiation from the skin of an animal, we find the difficulties which attend the subject even in the case of non-living matter much increased. We have no longer a radiating surface of definite and constant character. Not only may the difference of temperature between the skin and the environment change from time to time, but the physical condition of the epidermis itself may undergo variations, independent of or consequent upon, variations in the state of the corium. The factor c in equations (1) and (2) can no longer be assumed to be constant. Apparently from Masje's results (Virchow's *Archiv*, Bd. 107, pp. 17—71 and 267—290), the change in the emissive power of the skin may be so great, under certain conditions, as to mask and even to annul and reverse the effect of variations in the temperature. These conditions are, however, to a great extent abnormal. But in considering the part which radiation plays in the normal heat loss, we are met by the question, *What is the normal radiating surface in man and animals?* Is it really the naked epidermis? A little confusion on this point seems to exist in the minds even of some physiologists. Certain it is, that the enormous proportion of the heat lost by radiation to the total heat loss, which is observed when the skin is exposed in a physiological experiment, is not a normal ratio. Even in the case of the lower animals it can exist only under highly artificial conditions. In the first place only a small proportion of the naked epidermis is exposed under normal circumstances, and under the conditions which favour a rapid radiation. In man cooling by radiation is practically confined, outside the tropics, and in civilized races, to radiation from the clothes. In warm-blooded

animals it is confined to radiation from hair or feathers. In no case can the coefficient of emission of the real radiating surface alter much. It is again a non-living surface, whose properties may indeed be altered by physical changes in the air, but the radiation from which must be chiefly affected by changes in the temperature-difference between it and its surroundings.

But secondly, it is only when the body is at rest in still air that radiation can play a great part even in cooling the covering of the skin. When either the body or the air is in motion much of the heat lost must be given off by conduction and convection. Even a body with lamp-blacked surface, cooling in a closed space with lamp-blacked boundaries, loses only about half its heat by radiation, the other half being carried away by the air.

In normal circumstances, then, radiation is not the chief way in which the body loses heat. Further, what radiation there is is chiefly from surfaces whose excess of temperature is maintained by conduction from the epidermis, and the emissive power of which cannot alter much. A regulation of the heat loss by alterations in the amount of radiation cannot take place to any great extent by changes in the emissive power of the epidermis, cannot probably take place at all by alterations in the emissive power even of the physiological protective coverings. If such a regulation exists, it must be by means of changes in the conducting power of the epidermis, and of the temperature of the radiating surface.

This paper includes (1) observations on the temperature of the skin of man when covered and exposed, and on the temperature of the surface of physiological and artificial protective coverings in man and one or two animals; (2) measurements of the amount of radiation from the normal radiating surfaces, and from skin which is normally covered; and (3) simultaneous measurements of temperature and radiation.

Methods and instruments used.

The relation between the temperature and the resistance of a metallic conductor gives us the means of constructing thermometers and radiometers of great accuracy and sensitiveness, and

of late years the principle has been more and more applied in physical investigations in the domain of heat. It has also been introduced into physiology. Masje in 1887, in an elaborate research on radiation from the human skin, of which we shall have more to say later on, used a grating of tinfoil as radiometer; and quite lately Rolleston has attempted, with a negative result, to detect an increase of temperature in active nerves by means of a resistance thermometer of platinum wire. (*Journal of Physiology,* XI., 208–225). More than three years ago I made a similar attempt with a grating of gold leaf, prepared by fastening the leaf upon a large coverslip, and then cutting the grating out, the whole being covered with a thin layer of varnish. Six sciatic nerves of the frog were arranged on one grating and covered with another, the two being connected in series, and balanced by a similar pair. The result of the first experiments was, like Rolleston's, negative, but the work had to be broken off before enough had been done to warrant a definite conclusion, and it was never resumed. Before the gold leaf had been tried an attempt was made to produce a grating on glass by the process of electro-gilding, with fair success; but it was feared that the plumbago lines upon which the gold was deposited might affect the accuracy of the apparatus, and it was not used. In this work I have employed gratings of lead paper.

I. Measurement of the temperature of the radiating surfaces.

I do not know of any investigation of the temperature of the skin which has been made before with an apparatus of this kind; yet the method marks itself out as pre-eminently suitable for the measurement of the temperature of surfaces. What is required here is an instrument which can be accurately adjusted to the surface, which will quickly and certainly acquire the same temperature, and which at the same time will not sensibly alter that temperature by preventing the free radiation and conduction of heat. The resistance thermometer fulfils the first condition because it is easy to give

it any form. It fulfils the second, bcause its capacity for heat can be made extremely small by using very thin wire or foil, by which at the same time its sensitiveness is increased. It fulfils the third condition, both on account of its small capacity, and because it can be so arranged that the average temperature of an area can be found without covering more than a portion of it. It has great advantages over the thermopile because of its small mass, its plasticity of form, the quickness with which it comes to its position of equilibrium, and the fact that the resistance is approximately proportional to the temperature. Its greater sensitiveness is an advantage for some purposes, but not for measuring the temperature of the skin. It is of course no disadvantage, because it can be easily reduced. All this is true of almost any form of resistance thermometer. Another advantage, for work where extreme accuracy is not required, is that anybody who can measure a resistance can make himself a sensitive thermometer in a very short time out of the commonest materials. I used three forms in measuring the temperature of the skin. In making the first, a piece of ordinary lead paper was fastened to a coverslip with shellac varnish. It was then cut out into a grating, with a sharp pointed knife, each bar being about a millimeter in breadth, and the distance between two adjacent bars being about half a millimeter. The coverslip was fastened to a simple wooden holder, the grating covered with a slip of the thinnest glass, and the unscored ends of the lead paper were turned up round the holder, and tacked to it by a small copper nail on each side, which was soldered to copper connections. The resistance was between three and four ohms and the area of the sensitive surface a little more than a square centimeter. It would be perfectly easy to make the area very much less than this, but what I wanted to measure was not the very small differences of temperature which can exist in neighboring portions of the skin, but the average temperature of areas of moderate size.

This grating was balanced by another of precisely similar construction, the two forming the arms of a Wheatstone's bridge, which was completed by a graduated wire with a sliding contact.

Two methods of observation were used. In the first the bridge was balanced, one of the gratings applied to the skin (the same one was always employed), and the resulting deflection read off. This is porportional to the difference of temperature between the two gratings, and by an experimental graduation the value of the deflection in degrees is obtained, this value being the excess of temperature of the skin over that of the air. In the other method two equal resistances were introduced one at each end of the bridge wire. The resistances were of such amount that with the maximum difference of temperature between the gratings the slider had to be moved over three-quarters of the length of the wire in order to restore the balance. Instead of reading the deflection, the position of the slider with null deflection was read before and after the application of the grating to the skin. The experimental graduation gave the difference of temperature corresponding to a given difference of position of the slider. Experiments 1 and 2 are examples of the two methods. In using the first method it was necessary to be careful that the battery was always set up in the same way, so as to have a constant E.M.F. and resistance.

EXPERIMENT 1. Deflection read $\left\{\begin{array}{l}\Delta = \text{difference of temperature between the part and the air of the room.} \\ T = \text{temperature of the part.}\end{array}\right.$

Region.	Deflection.	Δ	T	Temperature of room.
Anterior surface of left forearm.	222	16·8	34·4	17·6
Posterior surface of left forearm.	218	16·4	34·0	17·6
Anterior surface of left arm over belly of biceps.	232	17·6	35·0	17·4
Left leg over head of tibia.	190	14·4	31·9	17·5
Skin just below xiphoid cartilage.	226	17·2	34·7	17·5
Skin over sternum.	206	15·6	33·2	17·6
Trousers over anterior surface of left thigh (nothing between trousers and skin).	82	6·1	23·7	17·6

EXPERIMENT 2. Position of the slider read.

Region.	Δ	T	Temperature of room.
Palm of left hand.	12·58	30·95	18·37
Forehead.	14·70	33·04	18·34
Right cheek.	14·92	33·21	18·29
Left breast.	16·01	34·40	18·39
Right hypogastrium.	17·00	35·16	18·16
Over apex beat.	16·32	34·57	18·25
Sole of left foot.	12·75	31·05	18·30

The second form of grating was designed to allow part of the area whose temperature was being measured to be still in free communication with the air, or perhaps more correctly to measure the average temperature of alternate strips of the given area. A lead paper grating was cut out on thin cardboard, the latter being cut clean away in the intervals between the bars of the grating. The breadth of an interval was the same as that of a bar. The surface was lightly varnished, and the whole mounted in a handle attached to one side. Experiment 3 is an example of the results with this grating.

EXPERIMENT 3. The measurements were made immediately after those of Experiment 2, on the same parts, and as far as possible under the same conditions.

Region.	Δ	T	Temperature of room.
Palm of left hand.	12·83	30·99	18·16
Forehead.	14·41	32·71	18·30
Right cheek.	14·63	33·07	18·44
Left breast.	15·93	34·18	18·25
Right hypogastrium.	16·82	35·07	18·25
Over apex beat.	16·68	34·68	18·00
Sole of left foot.	12·50	30·61	18·11

A third form of lead paper grating was devised to allow the outer surface of the metal to be freely exposed to the air. The grating was attached to a narrow frame of cardboad, and sup-

ported by a wide-meshed silk netting on its outer surface, while the surface next the skin was varnished. With care it was possible to get steady contact without injuring the grating.

Experiment 4 is an example. The measurements were made immediately after those of the last experiment.

Experiment 4.

Region.	Δ	T	Temperature of room.
Palm of left hand.	12·44	30·72	18·28
Forehead.	14·14	32·60	18·46
Right cheek.	14·87	33·00	18·13
Left breast.	15·91	34·01	18·10
Right hypogastrium.	16·56	34·83	18·27
Over apex beat.	16·12	34·42	18·30
Sole of left foot.	13·00	30·84	17·84

In each experiment the temperature of the parts usually covered by the clothes was taken immediately after exposure, and the results of such measurements are fairly uniform. Experiments 3 and 4 shew temperatures which do not differ much from those of Experiments 1 and 2; but on the whole the temperatures are somewhat lower in the former. This was found to depend not upon cooling due to previous exposure, but upon the kind of instrument used. The first form described reads a little too high, probably because the free radiation and conduction of heat is interfered with, and the evaporation of sweat checked, more than when the other forms are used. Still it is fairly accurate and suitable for every purpose of relative temperature measurement, although not so suitable as the others when the actual temperature of a part has to be determined to the second decimal.

But almost any form of resistance thermometer would be a great improvement upon the mercury thermometer for measuring skin temperatures. The great constancy of the readings for parts of the skin where the temperature has reached its stationary state, *e.g.*, the hands or face of a man who has sat for some

time at rest in still air, or a part of the usually covered skin which has cooled to its minimum, is a good proof of the reliability of the method.

When a part of the body which is normally covered is exposed to the air of a cool room the temperature declines progressively to a minimum, which, with small variations, it maintains. Experiments 5 and 6 are Examples.

EXPERIMENT 5.

	Time.	Δ	T	Temperature of room.
Anterior surface of left forearm exposed at 4.10....	4.10	15·23	33·43	18·20
	4.17	14·81	33·23	18·42
	4.23	14·10	32·66	18·56
	4.29	13·77	32·37	18·60
	4.37	13·11	31·71	18·60
	4.42	13·40	32.02	18·62
	4.55	13·40	32·02	18·62

The temperature remained constant at about 32·0 for 20 ninutes longer, when the experiment was broken off.

EXPERIMENT 6.

	Time.	Δ	T	Temperature of room.
Anterior surface of left forearm exposed at 5.5.....	5.4	—	—	—
	5.5	11·82	32·43	26·61
	5.15	11·04	31·64	20·60
	5.24	9·40	30·03	20·63
	5.83	9·65	30·25	60·60
	5.40	9·42	30·02	20·60

The cooling is not uniform over the whole of the exposed urface. In general the extensor surfaces of the limbs cool nore quickly than the flexor, but sometimes there may be little lifference. The decline of temperature is especially marked vhere there is no great thickness of muscle between the skin

and the bone, or where there are only tendons. The skin over the patella and shin cools rapidly, while the temperature of the back of the hand is nearly always less than that of the palm.

The temperature of the surface of the clothes in man and of the hair of animals is a very important element in determining the amount of radiation. It depends chiefly upon the temperature of the epidermis, the conductivity of the coverings, and the temperature of the air. It must be remembered that air when not free to move is a very bad conductor of heat, and the air between the clothes and the skin and in the pores of the clothes, and between the hairs and feathers of animals, is a most important protective covering. The aqueous vapour in the air also absorbs radiant heat from a low temperature source with great readiness, as Tyndall has shewn (*Phil.Trans.* 1861); and still air near the skin must be saturated or nearly saturated.

The difference of temperature between the surface of the clothes and the air will determine the amount of radiation and conduction, and it is certain that in man a great part of the heat regulation consists in keeping this difference approximately constant. If the temperature of the air falls, that of the surface must fall too, unless the loss of heat is to increase. Between the surface of the clothed skin and the outer surface of the clothes the slope of temperature must become steeper. Now the flow of heat by conduction is proportional to the slope of temperature and the specific conductivity, and inversely proportional to the thickness of the conductor. The flow must be quickened when the slope of temperature becomes steeper, the other factors remaining unaltered. But if the thickness of the conductor be increased or its specific conductivity diminished, the flow may be kept the same as before. This is, of course, what happens when additional clothes, or clothes of a warmer kind are put on in cold weather. When this compensation is not complete, the slope of temperature may be made less steep by warming the air artificially. When from any cause compensation cannot be obtained by variations in the factors governing the outflow of heat, the increased flow may be balanced by an increased production, due either to visible muscular contractions,

voluntary or involuntary, or to increased metabolism unaccompanied by such contractions. Heat regulation by alteration in the heat production seems to be more complete in the lower animals than in man (Loewy, Pflüger's *Archiv*, Bd. 45, S. 625). This is what we should expect, not only on account of the smaller size of the animals generally used for experiment, but because animals of any size have very little voluntary control over the difference of temperature of the radiating surface and the surroundings.

I have measured the temperature of the real radiating surfaces in a man clothed in the ordinary way and seated in a still atmosphere, and at the same time measured the radiation. The temperature of the room varied from 17° C. to a little over 20° C. The excess of temperature of the surface of the clothes varied from 6°·1 over the thigh, which was covered only by the trousers, to 1°·51 outside of the coat when it was buttoned up. Outside of the waistcoat it was in one experiment 4°·17. Between the waistcoat and the skin there was a linen shirt and a flannel undershirt. The coat sleeve over the fore-arm had an excess of 4°·32; over the upper-arm, of 3°·88. The excess of temperature did not vary much for a change of a few degrees in the temperature of the room.

The rest of the radiating surface, the skin of the hands, neck, and face, and the hair, had of course a much greater excess of temperature, ranging from 11°·61 on the palm and 10°·83 on the dorsum of the hand to 14°·54 at the side of the neck, and 13°·86 on the cheek, the surface of the hair having an excess of 7°·4.

The skin of a guinea-pig at one place had a temperature of 34°·50, the surface of the hair over it a temperature of only 29°·11; the skin at another place 35°·83, surface of hair, 29°·56; the skin of a rabbit, 36°·80, hair over it, 31°·54.

II. Measurement of the quantity of heat radiated.

Arrangement. I used at first a thermopile, but soon abandoned it for a lead paper grating fastened on a frame of stout cardboard, and blackened on one surface. It was balanced in the Wheatstone's bridge by a similar grating, and the measure-

ments were taken in the manner described for the temperature. The grating which received the radiation was of course kept at a constant distance (4½ centimetres) from the radiating surface.

The amount of radiation varied greatly at different parts of the surface. Over the clothes it was nearly proportional to the excess of temperature, as was to be expected, the outside clothes being all of the same material. The radiation from the exposed skin was also approximately proportional to the excess of temperature, but in a higher ratio, the skin having a greater coefficient of emission than the clothes (these were of light grey material). The total radiation under the most favourable circumstances did not exceed the rate of 700,000 calories in 24 hours for a body-weight of 70 kilogrammes. Taking the surface and, therefore, the radiation as proportional to the body-weight, this would give 820,000 calories for a bodyweight of 82 kilogrammes. Helmholtz has calculated the total heat loss from the skin, by evaporation, radiation, and conduction, for a man of 82 kilogrammes weight, at about 2,180,000 calories for a temperature of the air of 20°. If we substract 280,000 calories for evaporation, we get about 1,800,000 calories as the loss by radiation and conduction together; and of this the radiation would account for less than half.

Masje calculated from his results, that the quantity of heat radiated from the body of a man of 82 kilogrammes weight was 1,728,000 calories, which agrees, according to him, almost precisely with the calculated heat loss by the skin, exclusive of that due to evaporation. This leaves scarcely anything for conduction, which is certainly a mistake. It is beside the point to say that "the conductivity of still air is very small, almost 20,000 times smaller than that of copper." Under the conditions of his experiments and indeed under any circumstances in which the skin is exposed to the air of a room, conduction and convection must play a great part. The reason why Masje got such a high value for the radiation is not because the other portions of the heat loss were very small, but because the total heat loss in his experiments was very great, much greater than the normal. The agreement of his calculation for radiation

alone with Helmholtz's calculation for radiation and conduction together is only accidental. What Masje measured was the heat radiated from the *naked skin* in a cool room; and nobody can doubt that the total heat loss, with an air temperature of 10°—15° C. must be greater when the body is naked than when it is clothed. This is true, not only when the whole body is actually stripped, but also when the heat loss is reckoned from that of a number of limited areas, singly and successively exposed. If Masje had measured the total heat loss by radiation and conduction, under the conditions of his experiments, he would have found that the fancied agreement disappeared, and the disappearance of it would be a necessary proof of the correctness of his results. The mistake, however, can only be considered as a slip in a very thorough and scientific paper; but it is well to point out that Masje's whole investigation, interesting as it is, has only a limited application to the question of heat radiation from the body under normal conditions.

For example, in one of my experiments the heat radiated from unit area of the palm of the hand was to that radiated from the surface of the sleeve of a thin flannel shirt over the anterior surface of the corresponding forearm, as 204 to 42, or in round numbers 5 to 1. The radiation from the naked skin of the anterior surface of the forearm was to that from the same thin covering as 270 to 42, or more than 6 to 1, while its proportion to the radiation from the surface of the coat was as 270 to 28, or nearly 10 to 1. From the cheek the radiation was almost exactly six times as great as from the flannel covering the forearm. It is evident that if we were to take the radiation from the naked skin as the measure of the normal heat loss by radiation, the value would be far too high; for the clothes are warmed chiefly by conduction.

We have seen that there are two factors which may affect the amount of radiation, the temperature difference and the emissive power. Masje could not find any decided influence of the former on the radiation from naked skin; but he found that the emissive power varied in a very remarkable manner. He found that when the whole body or a large part of it was exposed to the

air of a cool room, the radiation increased with the time of exposure; and when the temperature of the room was only 9°—10° C., it might in less than an hour reach double or even quadruple its initial amount. He ascribes this to changes, perhaps partly produced reflexly through nervous influence, which alter the emissive power of the surface. I do not dispute the accuracy of the observations on which he rests his conclusions, although I am unable from my own experiments to confirm them, as I worked with a higher temperature of the air. It is to be expected that physical changes in the radiating surface, such as must take place in the skin both from physical and physiological causes, affecting notably the amount of moisture contained in its superficial layers and on its surface, should affect also the emissive power. And although the outer layer of the epidermis can scarcely be susceptible to direct nervous influence, yet it is not impossible that changes in the rete Malpighii brought about through efferent nerves acting directly on its cells may have the supposed effect. On the other hand there are so many known factors by which the amount of radiation, as measured by a pile or a bolometer, may be influenced, that it is only when these are obviously insufficient to explain a phenomenon, or when there is strong direct evidence that they are not connected with it, that we should call in the aid of "direct nervous action."

There is one factor which must affect all radiation experiments on the animal body, and especially on the human skin; and that is the quantity of sweat given off. Apart from its effect on the temperature of the skin, which will influence the quantity of heat emitted, the amount of watery vapour, and perhaps of other substances such as the aromatic bodies in sweat, in the layer of air next the skin, will affect the proportion of the radiated heat which reaches the recording instrument. The absorptive power of water vapour for radiant heat from a source at the temperature of the surface of the body is very great. Carbonic acid gas is also a very much better absorber than dry air. Certain organic vapours in very minute traces almost prevent radiant heat from passing. In

still air there must be a layer next the skin which contains more watery vapour than the atmosphere. When the sweat glands are active this layer will be of greater thickness and more highly charged with watery vapour, carbonic acid, and possibly with other substances which are even better absorbers of radiant heat, than when the secretion of sweat is slow or in abeyance.

If we take the quantity of water given off as insensible perspiration for an average adult, at, say, 650 c.c., it is easy to calculate the volume of dry air which would be saturated by it for any given temperature. One gramme of aqueous vapour can saturate, in round numbers, 33,000 c.c. of dry air at 30° C., and 650 grammes would saturate 21,450,000 c.c. Taking the surface of the body at 20,000 sq. cm., we get about 1,070 c.c. of air saturated at 30° C. per centimeter of surface in the 24 hours. This is equal to about $\frac{3}{4}$ of a c.c. per minute. In a minute the evaporation from the skin would suffice to surround the body with a shell of air, saturated for the temperature of 30° C., of three-quarters of a centimeter in thickness, if there was no movement of the air.

Séguin's estimate of the material given off by the skin at $\frac{1}{67}$ of the bodyweight in 24 hours would correspond to about double this thickness of saturated air at 30° C.; and to a saturated layer 3 centimeters thick at 20° C. For originally dry air at 10° C. the layer would be rather less than 5 cm. in thickness; and for air originally half saturated at 10° C., the thickness of the layer raised to saturation point in a minute would be 10 cm.

Of course we cannot assume that saturation does actually go on in still air so quickly as this. But if we consider that the average rate of evaporation for the 24 hours over the whole surface may be much exceeded at certain times and for particular parts of the skin; and further that aqueous vapour does not necessarily diffuse away at the rate depending on its density but may be condensed on dust particles in the air and re-evaporated, we shall see that some absorption of the heat radiated from the body must take place between the instrument and the skin, and that this will be greater the more freely the sweat glands are acting.

A small apparent increase in the radiation when the skin is exposed for some time in a room, with a temperature of 17° C. to 20° C. might be explained as due to diminished activity of the sweat glands, and therefore to diminished absorption. But the increase observed by Masje with a temperature of the air of 10° C. and 13° C. is too great to be thus accounted for, and the initial intensity of radiation does not seem to leave room for a very extensive absorption. Besides, a *warm* bath seemed in his experiments to cause an increase of radiation, even after the skin temperature had sunk to normal. I cannot say that I have been able to satisfy myself that the increase of radiation which undoubtedly follows the use of a bath a few degrees higher than the skin is due to anything else than the increase of temperature of the radiating surface. But this may again be due to the temperature of the room being higher than in Masje's experiments.

The effect of some antipyretics in increasing radiation certainly seems to favour the view, that the emissive power is increased; but when an antipyretic causes flushing of the skin it must increase the *temperature* of the radiating surface. Antipyrin, as Masje rightly remarks, reduces the temperature in the axilla, at the same time that it increases the radiation from the skin. But reduction in the temperature of the axilla or in the temperature of the blood, is not the same as reduction of the temperature of the naked skin. The radiation from a flushed skin must come partly from a layer as deep as the most superficial bloodvessels, else we should not see the flush; and doubtless rays of greater wavelength than the extreme visible red can pass through the epidermis.

The greatest difficulty in the way of the explanation of such immense changes in the intensity of radiation as Masje saw, by changes in the emissive power, is their very magnitude. The emissive power of the skin under *normal* conditions is high. I have found that a thin layer of lampblack does not much increase the radiation from the palm. In some experiments it even seemed to diminish it. But there is a possible fallacy here. Some of the radiation from the skin certainly comes from the

deeper layers. The lampblack will absorb this, and being at a lower temperature at first it will not give it all out again. But dead skin is also a good radiator. Two equal cubes of thin metal were covered with human skin. The skin covering one of them was coated with lampblack on the outside. That covering the other received a similar coating on the inside before being put on, so that the conductivity might be the same in both. The cubes were filled with water at 40° C., and allowed to cool. The rate of cooling showed that the lampblacked surface did not radiate much faster than the skin. But if the emissive power of skin can be increased fourfold, it must before the increase be less than one-fourth that of lampblack. Masje found that when the skin of the arm was cooled 3° C. to 4° C. below the normal the radiation fell off instead of increasing, and he explains this as due to the effect of the diminished temperature more than counterbalancing the effect of the increased emissive power.

It is difficult to see why if the emissive power can be increased fourfold by cooling the surface, a decline of 3° C. in the surface temperature should have any sensible effect in checking the radiation. From equation (1), p. 101, we see that the radiation will remain constant if c is increased in the same proportion as θ is diminished. Taking 3°—4° as the utmost by which the temperature of the skin can be diminished without diminishing the radiation, we find that an increase of one-fifth in the coefficient of emission would in Masje's experiments be enough to balance the fall of temperature. But if an increase of fourfold is possible, why is an increase of a fifth not forthcoming?

If we take equation (2), p. 101, we get for the radiation from skin at 31° C., with a temperature of the room of 15° C., the expression

$$R = c\,(a^{31} - a^{15}) = ca^{15}\,(a^{16} - 1),$$

and for the radiation from the skin at 28° C.,

$$R' = c\,(a^{28} - a^{15}) = ca^{15}\,(a^{13} - 1) \therefore \frac{R}{R'} = \frac{a^{16} - 1}{a^{13} - 1}.$$

Now $a = 1{\cdot}0077$, and $\frac{R}{R'} = \frac{1306}{1049}$, or about $\frac{5}{4}$.

If we take 4° as the fall of temperature,

$$\frac{R'}{R} = \frac{1306}{964}, \text{ or about } \frac{4}{3}.$$

So far as the temperature factor is concerned the radiation would only be diminished by $\frac{1}{5}$ for a diminution of 3° C.; and by $\frac{1}{4}$ for a diminution of 4° C.; again we are confronted by the question why the increase in the emissive power, which can assume such proportions when the temperature is lowered by 2° C., should be unable to compensate for the trifling diminution of radiation ($\frac{1}{15}$ or $\frac{1}{12}$ at most) caused by a further lowering of 1° C. Either the emissive power cannot alter so much as Masje thinks, or it is not altered in the same sense, or in the same proportion if the sense be the same, for a fall of temperature of the skin of 3° C. as for a fall of 2° C. It will not do to say that it reaches a maximum when the skin is cooled, say 2° C. It must decline from this maximum or possibly sink even below its original amount, if Masje's explanation is to be held sufficient. The greater the changes in the emissive power which must be postulated in order to explain his results with moderate cooling, the more difficult does it become to give a consistent explanation of what happens when the cooling is carried a little farther. Even if we suppose that the greater part of the radiation is from layers below the surface, and that the absorptive power of the superficial layers for this radiation is the chief variable, the difficulty is still to explain how so large a variation can be brought about, and why it should so abruptly change its sign. With an external temperature of from 17° C. to 20° C. I have certainly found that the temperature of the skin is a far more influential variable than the emissive power. Masje, on the other hand, working with lower external temperatures, found just the reverse. No entirely satisfactory explanation occurs to me of this difference — discrepancy it can hardly be called, because there is no reason why the factors which determine the amount of radiation should affect it in the same way with a low, as with a moderately high external temperature. The influence of the temperature factor is very clearly seen in parts of the

skin which are normally exposed, while the emissive power of such parts remains approximately constant. Experiment 7 shews the effect of various conditions of such a surface and of changes of its temperature on the radiation. When the temperature of the hand is raised or lowered by immersion *for a short time* in a bath, the radiation is correspondingly increased or diminished. This contrasts with the behaviour of the normally covered skin as observed by Masje, who found that cold baths might greatly increase the radiation.

EXPERIMENT 7. Palm of left hand.

Time.	Condition of Surface.	Deflection.	
11.30 a.m.	Moist with sweat.	100	The temperature of the room during the observations varied from 20° C. to 20°·3.
11.32 "	Dried lightly with cloth.	130	
11.36 "	Washed in water at 20° and dried.	122	
12.30 p.m.	Felt cool.	98	
2.30 "	Immediately after walking—felt warm.	121	
2.40 "		126	
2.45 "	Covered with vaselin.	86	
2.50 "	Still covered with vaselin.	78	
2.55 "	Vaselin washed off—hand dried without rubbing.	126	
2.59 "	Palm covered with lamp-black.	101	
3.2 "	" "	105	
3.4 "	" "	112	
3.6 "	" "	98	
3.9 "	Lamp-black removed.	120	
3.10 "	" "	124	
3.15 "	Rubbed briskly with towel—felt very hot.	158	At 3.18 the radiation had sunk to 123.
3.20 "	Heated in dry air at 50° C. for 1 minute.	148	
3.25 "	After being 1 minute in bath at 19° C.; dried without rubbing.	96	
3.30 "		86	
3.35 "	After being 1 minute in bath at 27° C.	99	
3.40 "	After being 2 minutes in bath at 36° C.	134	
3.50 "	After being 2 minutes in bath at 46° C.	168	At 3.45 radiation was 125.

A division of the scale represents about ·000006 small calories (gramme-degrees) per second per cm. of surface.

III.—SIMULTANEOUS MEASUREMENT OF THE SURFACE TEMPERATURE AND THE RADIATION.

I do not propose to do more here than to describe the method of making the observations and to give a single example of

hem, as I hope to have an early opportunity of returning to he subject in connection with some calorimetrical work.

Both the temperature and the radiation were measured as efore by the resistance method, and the radiometer was the ame lead paper grating as was previously used. The thernometer applied to the skin consisted of a narrow strip of varished lead paper, forming the sides of a square whose internal rea was equal to that of the sensitive part of the radiometer. t was attached to the radiometer at a distance of 4½ centimeers; so that when a part of the surface of the body was aplied to the thermometer it was in the proper position for the easurement of the radiation. The instruments, balanced by wo precisely similar arrangements, were each introduced into Wheatstone's bridge, the two systems being quite separate nd the galvanometers independent. The deflections were imultaneously read. Strictly speaking it was not the temperture of the radiating surface itself which was measured, but at of its boundary, which must be approximately the same hen the area is not too large.

EXPERIMENT 8. Palm of right hand.

Condition.	Radiation.	Δ	T	Temperature of room.
ormal.	107	10·40	31·0	20·60
rm held above head for 2′.	70	9·05	29·65	
and hanging down for 2′.	128	11·1	31·7	
and kept at level of heart for 2′.	120	10·4	31·0	
and round wrist so as to prevent the venous return.	121	10·9	31·4	20·5
and immediately after bath at 18° for 2′.	90	9·50	30·11	20·61
and after brisk rubbing.	152	14·07	34·57	20·50
and after bath at 33° for 2′.	112	10·84	31·34	20·50
and after bath at 45° for 2′.	174	15·10	35·52	20·42

Δ means, as before, the excess of temperature of the skin over the air.
T the temperature of the skin.

It will be seen that the radiation from the palm is in most

cases approximately proportional to the temperature; and therefore the coefficient of emission for the palm may be looked upon as fairly constant. This does not apparently apply to the normally covered skin, nor to the skin in fever. Masje states that in fever the radiation is less than the normal, although the temperature of the skin is greater. This can scarcely be true of the whole heat loss by the skin, for, in fever, after the stationary temperature has been reached, the loss of heat must equal the production, and the production is increased. More heat must therefore be lost in other ways than is normal; by conduction and convection and by respiration that is to say.

The general conclusions to which I have been led are that under normal circumstances the heat loss cannot be regulated by alterations in the emissive power of the radiating surface either in man or animals, and that, for the external temperatures with which I have worked, no marked change in the emissive power of the human skin can be brought about by heating or cooling it. The difference of temperature between the radiating surface and the environment is therefore the chief factor which affects the heat loss. When this is increased the heat loss is in general increased, whether the greater difference of temperature be brought about by lowering the external temperature, or by increasing that of the radiating surface, or by substituting for the normal radiating surface another of higher temperature, or greater emissive power, or both.

When a large part of the skin of an animal which is normally protected by an artificial or natural covering is exposed to air at a temperature of 15° C. to 20° C., the loss of heat by the skin is increased; and it depends (*a*) upon the size of the body, (*b*) upon the perfection of the heat-regulating mechanism, whether the internal temperature falls or not. The larger the body the less must the proportional increase of the heat production be, in order that the internal temperature may remain constant.

In small animals, such as rabbits and guinea-pigs, it is astonishing how little an increased heat production can supply the loss of a considerable part of the protective covering, or make up for an alteration in it which increases the outflow of heat. It has long been known that a rabbit dies when its skin is varnished.

'he superstition still lingers in some text-books that this is due ɔ interference with the excretory functions of the skin. This xplanation may perhaps hold good in the case of animals like ıe horse. It is not necessary for the case of the rabbit and ıe guinea-pig. For, without varnishing at all, if the hair be reıoved from the greater part of the body of either of these aniıals by rather close clipping or still better by shaving, and the nimal be kept in an empty box so that it cannot cover itself, it ies in summer weather with an air temperature of 15° C. to 8° C., and that sometimes in 20 to 30 hours. The rectal temperture sinks fast, notwithstanding almost constant involuntary ıuscular contractions. If the radiation from the skin be measred, it is found to be far in excess of that from the hair. The annal is evidently losing heat more rapidly than it can produce it. he heat regulating mechanism is overmastered by the sudden ıcrease in the heat loss from the skin. If now, before the cooling ıs gone too far, the animal be placed in a warm chamber, the ɛctal temperature rises again, and it recovers completely. The ıcrease of heat production can only respond within somewhat ırrow limits to an increased heat loss; and it would seem that the heat production once begins to lag behind the heat loss, if considerable diminution of the temperature of the blood has tually taken place, it becomes more and more difficult to reore the balance.

When the skin is varnished the same kind of thing goes ı. The hair owes its low conducting power chiefly to the large nount of air which it keeps at rest around the animal. When it varnished over, a great part of this air is expelled, the hair is ıttened down on the skin, and the escape of heat is accelerated. ıe dilatation of the vessels which is said to take place under the ırnish would increase this still more.[1] Possibly the emissive ɔwer of the varnish may be greater than that of the normal ıdiating surface. I have not, however, found this the case for e skin of man. At any rate, an alteration in the normal radiag surface, whether produced by varnishing or by removal of e hair may be sufficient to baffle any attempt at making up e increased loss by increased production, and the lower the ternal temperature the more easily will this take place.

[1] Any direct or reflex "tonic" effect which the actual contact of the air may ve on the skin is of course lost.

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THE CONDUCTIVITY OF ERYTHROCYTES COMPARED WITH THAT OF SERUM

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In 1896 I discovered, while making experiments on the output of the heart by a method involving measurements of the conductance of blood, that the specific conductivity of defibrinated blood and of sediments of blood was much less than that of the serum (15 or more times less in the case of sediments) and concluded that the cells (erythrocytes) were extremely poor conductors in comparison with the liquid in which they are normally suspended. This conclusion was at once extended to embrace leucocytes (pus cells), and the cells of various solid organs from which more or less satisfactory suspensions could be obtained (scrapings of intestinal mucosa, liver, spleen, thymus, etc.). The generalization was ventured upon that this was a property common to all cells. In my first two papers (Stewart, 1897a, b) the causes and some of the implications of this property of cells were discussed. It was pointed out (Stewart, 1897b) that since some serum is necessarily present in the sediments the conductivity of the erythrocytes must be much less than the actually measured conductivity of the sediments, so that, "in comparison with serum the conclusion seems warranted that erythrocytes are non-conductors" (Stewart, 1897a). Two alternative (not mutually exclusive) explanations were suggested: 1. That the corpuscles, while capable of electrolytic conduction in their interior, are surrounded by a (relatively) non-conducting envelope or external layer, impermeable, or little permeable, to certain of the ions of blood, but not necessarily structurally differentiated from the rest of the corpuscle. 2. That throughout the substance of the corpuscle the electrolytes are combined with proteins and hemoglobin, or at least that they are hindered from dissociating normally by the presence of these substances and their ions from migrating as they would in an aqueous solution. Some evidence was brought forward that compounds of the nature suggested could be liberated from erythrocytes by certain hemolytic processes. For instance, when blood is laked by repeated freezing and thawing, the conductivity is not usually increased but may be somewhat lessened; but if distilled water is now added to the already

ked blood the conductivity is increased just as if the water had been iginally added to unlaked blood. Emphasis was laid on the bearing of e observations on some of the well-known electrical phenomena exhibited such tissues as muscle and nerve (the difference of apparent conduct-ice in the longitudinal and in the transverse direction, electrotonic rrents, and other so-called polarization phenomena, the high degree of larizability of these tissues compared with the polarizability of the rface of contact of ordinary solutions of electrolytes, etc.).

Observations were also mentioned (Stewart, 1897b) on the conductivity gastric juice, milk and curds, pus, cyst liquids, etc. It was pointed out tewart, 1897b) that a relation exists between the ratio of the conduc-ities of blood and serum and the proportion of serum and erythrocytes in e specimen. A simple formula was given which is approximately correct r blood rich in serum or diluted with a known porportion of serum. hen the proportion of erythrocytes increases another factor than the iction of the total volume occupied by erythrocytes influences the con-ctivity, namely, the fact that conduction must take place along an "out d in" path, formed by the thin threads of serum twisting among the rpuscles. The conductivity therefore diminishes more rapidly than in oportion to the increase in the fraction of the total space occupied by e corpuscles as the content of erythrocytes is increased. The curve g. 1) ceases to be approximately linear and becomes convex towards e abscissa axis. The relative volume of serum and erythrocytes esti-ited by the conductivity method was compared with that estimated by colorimetric method (hemoglobin). In a later paper (Stewart, 1899b) ther extensive data were derived from accurately measured dilutions of ythrocytes suspended in their own serum. The proportions of serum ried from nearly 90 per cent to about 10 per cent. From these data it is shown that excellent determinations of the relative volume could be tained under conditions which left erythrocytes and serum entirely altered. I have regretfully observed that most writers seem to think at the chief interest of the fundamental fact that cells have a low con-ctivity lies in the application of it to the measurement of the concentra-n of the erythrocytes. This application is interesting and useful, but e physico-chemical structure of cells on which their low conductivity pends has far wider relations and a much greater biological interest than is. I have, at various times, discussed certain phases of this question tewart, 1899a, 1901, 1909, etc.), and have published numerous experi-nts in which erythrocytes and other cells, acted upon by substances ich cause hemolysis, leucolysis, etc., particularly those whose mode of ion is more or less understood, have been examined as to the changes oduced in their electrical conductivity, osmotic pressure and other operties. The effect of partial and complete fixation by various reagents

upon the conductivity has also been investigated in numerous instances (Stewart, 1902a, b). Results of considerable interest have been obtained by the study of the action of hemolytic, leucolytic and other substances upon the conductivity, etc., of partially and fully fixed cells. Microscopic study and enumeration of the "shadows" and measurement of their volume and the conductivity of suspensions of them in many forms of hemolysis, and with large and small doses of the active substances, have been carried out (Schroeder and Stewart, 1925). Incidentally, the so-called reversal of hemolysis (Brinkman et al., 1923–4) has been shown to be based on misinterpretation of certain appearances, as was indeed demonstrated by me (Stewart, 1902a, b) partly in association with Peskind (1902) many years ago. It is unnecessary to mention other directions in which the significance of the small conductivity of cells was explored by me, sometimes in collaboration with pupils. They are all to be found in easily accessible journals.

Conductivity of blood sediments. In the present paper I shall give data on the values of the ratio $\frac{k_1}{k}$ in sediments of blood centrifuged for shorter or longer periods, as a rule at a constant speed and in the same centrifuge. The specific conductivity of the sediment is represented by k and that of the serum by k_1. In some of the experiments one tube was removed after a certain number of hours and the conductivity of sediment and serum measured. Another tube of the same blood was taken out after a further period in the centrifuge. All the blood specimens were obtained from rabbits, defibrinated and immediately placed in the centrifuge. Rabbits were chosen chiefly because of the relatively large proportion of serum and the apparent ease of sedimentation. In every case the serum was completely free from hemoglobin. The ratio $\frac{k_1}{k}$ was determined for the defibrinated blood as well as for the sediment. The method of obtaining the sediment as free as possible from supernatant serum was as follows. All the serum which could be removed safely without admixture with any corpuscles was pipetted off. Then pipetting was carefully continued of the chief part of the remaining visible serum with small amounts of erythrocytes from the top of the sediment. Rolled up Swedish filter paper was then put down into the centrifuge tube till it touched the top of the sediment, left there for a couple of minutes, and then withdrawn. This was repeated about three times. The wall of the tube was kept clear of erythrocytes as far as possible by swabbing gently with filter paper. During this time the tube was in the bath, supported in a small copper gauze vessel, so that the sediment was practically at bath temperature (about 25°C.) when the conductivity tube was filled. This is important since it is certainly difficult, and possibly harmful to some of the erythro-

cytes, after the long centrifugation, to stir the extremely viscous sediment. With only one to two per cent of serum in the sediment, there may be 20,000,000 erythrocytes in the cubic millimeter. There is no need for stirring, as settling of erythrocytes in such a sediment is a process more theoretical than real. In any case the measurement is taken within a minute of the filling of the conductivity vessel, and this can be done safely if the material is already at bath temperature, as mentioned. The same is true of defibrinated blood or any dilution of erythrocytes: if the stock is already at bath temperature no stirring of the blood is necessary unless a series of observations extending over several or many minutes is required. Then, of course, the blood must be stirred. In ordinary defibrinated blood settling begins, of course, at once; in the first minute the erythrocytes settle as fast as, perhaps faster than in any other minute. But it can rarely, if ever, happen that this will introduce a sensible error if the blood is already at bath temperature and the measurement is made immediately after the tube is filled. Occasionally I have filled the conductivity vessel by pouring from the centrifuge tube the sediment, freed as much as possible from supernatant serum as described. Generally, however, another method has been preferred. A straight pipette with a wide bore has the point cut off. The pipette is closed by the finger to prevent the sediment, as much as possible, from entering it as it is passing through the upper layer of the sediment. The pipette is then introduced to about the bottom of the centrifuge tube and sediment sucked up. The blood is quickly rubbed off the outside of the pipette with a piece of filter paper and the conductivity vessel filled.

Some data are assembled in table 1 and plotted in figure 1. In the graph the first 22 points (down to the values of p, 11.5; $\frac{k_1}{k}$, 16.47 inclusive) were determined by actual measurement of the number of cubic centimeters of serum present in 100 cc. of the blood specimens. The numbers plotted as ordinates represent the cubic centimeters of serum. As abscissae are plotted the ratios of specific conductivity of serum to that of blood $\frac{k_1}{k}$. A very much larger number of points could have been inserted from the available data, without altering the curve at all. But it was desired not to overcrowd the graph so that it could be used for determining by means of a ruler the quantity p when k_1 and k were measured. The remaining part of the graph represents the continuation of the curve to the right on the same scale for values of $\frac{k_1}{k}$ up to 127.3 inclusive. This is the highest value of the ratio actually measured, the sediment having a conductivity over 127 times less than that of the serum. As some serum is necessarily included in the sediment, the conductivity of the erythrocytes

is certainly less than this. The values of p are calculated by means of formulae which fit the upper portion of the curve very closely, but the actual values of p have not been directly determined for the experiments included in table 1 and plotted in the six strips in the lower part of figure 1.

TABLE 1

NUMBER OF ANIMAL	SEDIMENT						SERUM	DEFIBRINATED BLOOD						
	K (25°) $\times 10^4$	$\frac{k_1}{k}$	Cubic centimeters of serum in 100 cc. (a)	(b)	(c)	F	K (25°) $\times 10^4$	K (25°) $\times 10^4$	$\frac{k_1}{k}$	Cubic centimeters of serum in 100 cc. blood (a)	(b)	(c)	F	Hematocrit
1	3.0	42	4.2	4.2	4.1	5.0	125.6	71.6	1.75	73.4	73.7	72.0	71.8	60 (45 m.), 63 (75 m.), 66.5 (125 m.), 67 (215 m.), 68.5 (365 m.)
2	1.6	80	2.1	2.2	2.0	2.4	126.7	62.7	2.03	67.1	66.4	64.9	65.0	60.5 (105 m.), 61 (150 m.), 62 (240 m.), 62.5 (330 m.)
3	1.15	111	1.6	1.6	1.6	1.7	128.7	63.7	2.02	67.6	66.8	65.2	65.2	49 (35 m.), 64.5 (95 m.), 65 (135 m.)
4	5.9	22.4	7.6	7.6	8.0	9.2	131.3	64.5	2.03	65.7	66.4	64.9	65.0	
5	1.5	88.6	1.9	2.0	1.9	2.1	132.0	64.8	2.04	65.9	66.3	64.8	64.8	60.5 (60 m.), 62.5 (150 m.), 63 (300 m.)
	1.0	127.3	1.3	1.4	1.3									
6	1.3	103.6	1.7	1.7	1.7	1.8	131.3	58.0	2.26	61.5	61.4	61.2	60.3	
7	1.1	123	1.4	1.4	1.4	1.4	133.4	65.4	2.04	65.7	66.3	64.8	64.8	59.5 (70 m.), 61.5 (135 m.), 62 + (200 m.), 62.0 (255 m.).
8	3.2	39.7	4.3	4.3	4.3	4.7	131.3	74.6	1.76	72.6	73.1	71.7	71.6	
9	3.3	38.2	4.5	4.5	4.5	4.9	126.1	71.6	1.76	73.4	73.1	71.7	71.6	
10	4.2	30.0	5.7	5.7	5.7	6.2	126.0							

Formula (a): $p = \frac{k}{k_1}(174 - k(5°))$. Formula (b): $\frac{k_1}{k} + \frac{1}{2} = \frac{174}{p} - \frac{3}{100 - p}$. Formula (c): $\frac{k_1}{k} + \frac{1}{2} = \frac{175}{p} - \frac{p}{400}$. The number of cubic centimeters of serum in 100 cc. of blood or sediment is represented by p. In column F are the cubic centimeters of serum calculated by Fricke's formula. Formula (c) can also be written $\frac{k_1}{k} + \frac{1}{2} = \frac{7 - p^2}{4p}$, where p is the proportion of 1 cc. of the blood which consists of serum.

If, however, these strips are imagined to be successively joined on to each other towards the right at the level of the abscissa axis of the upper part of the curve, it is seen that the curve is continued quite smoothly down to the value $\frac{k_1}{k} = 127.3$; the curve asymptotically approaching the

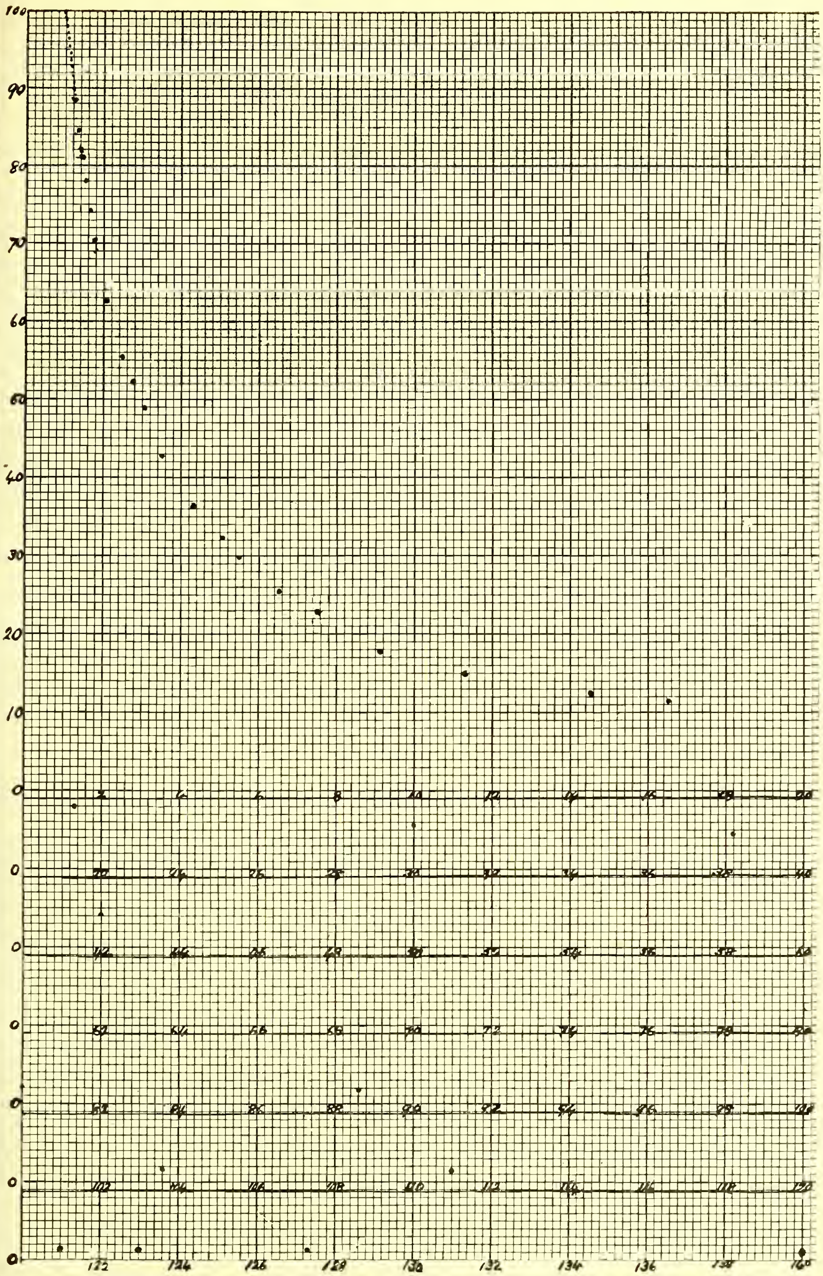

Fig. 1. Graph showing relation between the ratio $\frac{k_1}{k}$ (conductivity of serum divided y conductivity of blood) and p, the number of cubic centimeters of serum in 100 cc. f blood.

abscissa axis. One point, corresponding to $\frac{k_1}{k} = 140$, has been extrapolated, the value of p being calculated at 1.1, and 1.2 by my formulae (b) and (c) respectively, and at 1 by Fricke's formula (1924a, b). It cannot be assumed that a value of $\frac{k_1}{k} = 140$ or more will be found in a sediment obtained by centrifugation from defibrinated blood. But it is unlikely that I attained optimum conditions. It is, however, already clear that the conductivity of erythrocytes is very low compared to that of serum; perhaps hundreds of times less if we consider the relatively great effect which the presence of even a trace of serum would have. One writer (Brooks 1924–5) has thought it worth while to state that he assumes that erythrocytes have a small conductivity. The statement is not based on any measurements, but it is perfectly safe although not important, since the purest distilled water has a small conductivity. I cannot imagine anybody with the slightest claim to write on this subject supposing that these cells are absolute non-conductors. In the case of my data, where the proportion of serum was determined directly, the conductivity of the erythrocytes is not involved since the conductivities of serum and blood in the unknown specimen are simply compared with those of the standards in which p has been directly determined. The same is true of polarization, which was discussed in my first paper. Whatever polarization is involved in the apparent resistance of the unknown blood is also involved in that of the standard in which p is directly estimated.

It is probable that the maximum value obtained for $\frac{k_1}{k}$ can be increased and the minimum value for p diminished by improving the conditions under which the blood is centrifuged. A lower room temperature would be favorable. The optimum speed and duration of centrifugalization could be worked out by check experiments. I have found that if specimens of the same blood are centrifuged at a given speed for different periods it is not necessarily true that the sediment from the blood which has been longest in the centrifuge will have the lowest conductivity. Beyond a certain point the appearance of a small content of hemoglobin in the serum and diminished viscosity of the sediment indicate that the conductivity will be found much higher than that of the specimen centrifuged for a shorter period and whose serum contains no blood pigment. An instance is given in table 1, rabbit 7. Here $\frac{k_1}{k}$ was 123 for a specimen centrifuged 7 hours, and only 9.04 for a specimen centrifuged 13 hours. The second specimen was slightly laked, the supernatant serum being tinged with blood pigment. The temperature of the tubes was such that heat laking could not have occurred. The viscosity of the sediment was much less than in the first

pecimen. The great increase in the conductivity of sediments by the ddition of a trace of saponin, which causes complete laking, was illustrated n a previous paper (Schroeder and Stewart, 1925). The conductivity n one case rose from K (25°) $\times 10^4 = 3.0$ to 62.8. In blood from rabbit , table 1, the conductivity of the sediment increased from K (25°) $\times 10^4$ = 1.1 to 16.7 on addition of a trace of saponin.

It is easily seen from inspection of the curve, or of the numerical data rom which it is constructed, and of other data from which other almost dentical curves could be plotted (see especially, Stewart, 1899b) that there s no reason to expect a discontinuity at either end. The curve is perfectly mooth. Interpolation can be made with certainty at any point within he extreme observed values. It is evident, but has been indicated by a roken line, that extrapolation is possible at the top of the curve beyond he extreme observed value of p (about 90). In fact the broken part has een put in by laying a ruler so that the edge includes 5 or 6 of the first oints, where the curve is sensibly linear, and carrying on the line till it uts the ordinate represented by unity, corresponding to the ratio $\frac{k_1}{k} = 1$ i.e., $p = 100$). It should be noted that this was done on a graph which as been reduced to two-fifths in the figure. Whether the extrapolated art of the curve runs exactly into the ordinate 1 at the level of $p = 100$ or ot is of no consequence; it certainly comes very near to this.

Yet one writer (Brooks 1924–5) concludes after a so-called "critical examination," *lucus a non lucendo*?) without any experimental observations whatever, that my ork was "unsound" because, according to him, substitution of the extreme values $_1 = k$ (corresponding to 100 per cent of serum) and $k = o$ (corresponding to 100 per ent of erythrocytes), in my formulae led to impossible results. In fact, as anyone ith even a moderate acquaintance with elementary algebra ought to have seen, hen $k = o$ all the formulae used by me give the result $p = o$. This is also true of ricke's formula, where substitution of $k = o$ gives $\rho = 1$, i.e., 100 per cent of erythro- ytes. Of course the substitution is made after a slight transformation of the equa- ions. For instance by inverting the two sides of the equation k can be put into the umerator instead of the denominator. The same is true if $\rho = 1$ is substituted in ricke's formula. This is one of the ordinary devices when dealing with functions hich contain such a quantity as $a - x$ in the denominator of a fraction, if the value is to be given to x. It is common to find a section on indeterminate functions in extbooks of algebra, explaining how to deal with such expressions by artifices like he one mentioned, by differentiation and in other ways. In solving the quadratic quation left after substitution of a numerical value for $\frac{k_1}{k}$ in formula (b) the negative quare root is chosen. It is humiliating to have to mention these things in a scientific aper.

There is nothing disreputable about empirical formulae. They have often proved xtremely useful. Mine are a little more than empirical for it was clearly seen in onstructing them that they must be built around the ratio $\frac{k_1}{k}$ or its reciprocal. The

results deduced from them agree excellently with the experimental data and also with those yielded by Fricke's (1924a, b) theoretically derived formula. I agree with Brooks when he praises Fricke's work. It is perhaps at this date scarcely a breach of confidence to say that I was asked by the editor of the Physical Review, of course without Doctor Fricke's knowledge, to go over Fricke's paper for that journal (Fricke, 1924b) mainly, I suppose, because he had used my experimental data for testing his formula. The only thing I pointed out as requiring consideration was the fact that the ratio $\frac{a}{b}$ (diameters of the erythrocytes), which is involved in the determination of the constant β, was difficult to measure with any exactness and that, as a fact, our knowledge of the precise shape and magnitudes of these cells in different mammals left much to be desired. I noted, however, that Fricke's graphs showed that a considerable variation in $\frac{a}{b}$ could occur without causing a very large change in β. Brooks has given a good analysis of the uncertainties involved in measuring the diameters of erythrocytes. If I am not wrong, Fricke at present verifies his constant β to some extent by comparison of the results calculated from the formula with the experimental results. This is generally considered to be legitimate.

To return to my formulae, they were adapted, in the first instance, to fit the range of values which might be encountered in blood in health and disease. They really do more than this, but that was considered the essential thing. That a formula should give a correct value where the blood is all serum is of no importance at all for the practical purpose of determining the relative volume of corpuscles and serum in a specimen of blood, and has nothing to do with the "soundness" of the formula. We do not need to tell from a formula that we are dealing with pure serum. In fact formula (a) gives very good results up to 93 or 94 per cent. It is usually a few per cent too low when $\frac{k_1}{k} = 1$, i.e., for pure serum but not always. Formula (b) gives a close agreement up to about 90 per cent of serum. It is not difficult to construct a formula which shall give a correct result ($p = 100$) when $k_1 = k$, and also a correct result ($p = o$) when $k = o$. Formula (c) is such a formula. But in the important range there is no reason to think it any better than the other and that is why results calculated from (c) have been placed in table 1 for comparison. If there is any mathematical fault to find with (b) it is that the equation is of the third degree, whereas the other two are equations of the second degree. But as regards the practical use of this formula that makes no difference. I shall repeat here what has already been said that we can get along very well without any formula, by using the numerical data or the curves which bring us close to the underlying relations. Many instances could be given of empirical formulae fitting only a portion of a curve. For example, Regnault needed three formulae to fit different ranges of temperature for interpolating values of vapor pressure of water. Much later these three formulae were reduced to one.

Brooks' statement that my formulae "are unsound, since they give impossible values for conductance of cells alone or suspending fluid alone" may now be dismissed as based on nothing more than his inadequate knowledge of the subject.

Comparison of electrical method with hematocrit. As in all my previous work, I find that the relative volume of erythrocytes given by the electrical method is somewhat less than that yielded by centrifugation. This is true whatever formula is used, whether Fricke's or mine, as they all give

ιpproximately the same values. It is scarcely necessary to say that if the ⁄alues are interpolated in the tables of experimentally determined results ›r by measurements on the graphs, the same discrepancy is found. It s indifferent whether the hematocrit is used or the ordinary centrifuge ,ubes, both give the same result. Generally the discrepancy is 2 to 3 cc. n the sediment from 100 cc. of blood, the value of the sediment being ısually 5 or 6 per cent too great and that of the serum about 2 per cent too mall. Occasionally, the discrepancy is greater, and this is more likely o occur in specimens rich in corpuscles, and in which for one reason or ,nother the corpuscles settle with unusual slowness. Rarely the hemato- rit gives practically the same result as the electrical method, as in experi- nent 3, table 1, for the defibrinated blood. The reason for the discrepancy s that the electrical method gives the correct values. It cannot fail to lo so if the standard estimations have been made correctly, since the rythrocytes are unaffected by the measurements and are suspended ıormally in the serum. The hematocrit is by no means an instrument of ›recision, but it is quite good enough for most clinical observations if used ›roperly; and although the electrical measurements are simplicity itself, it s far better that persons who do not know how to make them accurately, r what to do with them when made should use the centrifuge. In table 1 nder "hematocrit" are given successive values for serum as centrifuga- ion proceeded; in means minutes from beginning of centrifugation.

The hematocrit does not yield entirely accurate values for the sediment or more than one reason. A factor which certainly contributes to the iscrepancy is the serum, which cannot be got rid of completely from the ediment. Nobody, I think, will have the hardihood to assert that the ediment in the centrifuge tubes consists of dried corpuscles, or even of orpuscles whose surfaces are dry. Dried erythrocytes lake even when laced in their own serum. This was shown long ago by Stewart (1902b) nd by Guthrie (1903). If the (rabbit's) erythrocytes are assumed for implicity to be discs 6μ in diameter and 2μ thick, about 18,000,000 would e contained in 1 cu. mm. of well centrifuged sediment. A layer 0.01μ hick around an erythrocyte would have a volume of about $0.95\mu^3$. For 8,000,000 erythrocytes the total volume of the layer would be about 7,000,000 μ^3 or 0.017 cu. mm. of serum in 1 cu. mm. of sediment, i.e., 1.7 er cent of serum. This is about the proportion calculated as present ı well centrifuged sediments in table 1. A thickness of 0.01μ would be ne-three hundredth of the radius of the disc-shaped corpuscle or about 30 mes the diameter of a molecule of carbon dioxide (Nernst). The layer f serum in contact with each erythrocyte on the assumption made is ccordingly very thin. If it were 5 times as thick, i.e., 0.05μ, the volume f the layer would be 0.082 cu. mm. in 1 cu. mm. of sediment, or 8.2 per

cent of serum. This is approximately the proportion of serum calculated for the least well centrifuged sediment in table 1.

Access of such quantities of oxygen as the cells in a sediment could use up if conditions for oxygen use were normal, and exit of such quantities of carbon dioxide as they produce must be rendered difficult or impossible in a tightly packed sediment in the bottom of a centrifuge tube. The sediment is seen to be dark after prolonged centrifugation. This factor and others which may influence the blood gases in the sediment may cause changes in the volume of the erythrocytes, although it is impossible to say what share, if any, they take in producing the discrepancy between the hematocrit and the electrical method.

There is of course deformation when the corpuscles are packed down by long centrifugation. Nobody knows whether this is associated with an increase in average volume due to the taking up of liquid. A change of this kind might be a factor in the observed, small discrepancy. In any case, if deformation occurs the relation between surface and volume will be altered, unless the change is balanced by migration of water. Some hemolysis can be caused by prolonged centrifugation when precautions have been taken against such rise of temperature as would cause heat laking. This has been referred to already in connection with the conductivity of sediments.

In obtaining sediments for making the dilutions in my standards for conductivity determinations only moderate centrifugation was employed, 10 per cent or more of serum being left in the sediments. It was not necessary to centrifuge exhaustively because it was desired to produce only such erythrocyte concentrations as could exist in blood. A check on the sediment dilutions was also afforded by the numerous observations on uncentrifuged defibrinated blood, in which of course there was no possibility of any change in the erythrocytes. The range of concentration of erythrocytes in the specimens of blood examined is nearly as wide as for the dilutions of sediments. Curves of the same kind can be plotted from the results on blood. They coincide closely with those plotted from the data on sediment dilutions and fit the formulae as closely. It can therefore be assumed with confidence that my standards were not sensibly affected by changes in the volume of the sediments due to deformation of the erythrocytes by centrifugation.

"Critical examination" of my experimental data. Brooks tries to show that my results understate the volume of erythrocytes contained in the various dilutions. He does not bring forward any experimental data to check up with mine. He simply sits at a desk and writes "If." "If," he says, in my estimations by a colorimetric method of the residual serum in the sediments with which I start in making the various dilutions by the addition of measured quantities of serum from the same blood, some of the

hemoglobin employed passes into the erythrocytes, the serum volume in the sediment will be overestimated and the erythrocyte volume underestimated. He gives no evidence whatever that normal erythrocytes take up hemoglobin, but cites Brinkman's (1923–4), observations on pseudo-reversal of hemolysis as showing that shadows after laking can do so. This is called a critical examination. Brinkman's conclusion is due, as already mentioned, to misinterpretation of certain microscopic appearances and is without foundation. But supposing it was true, how could it be assumed from this that intact erythrocytes loaded with the normal stock of hemoglobin would absorb hemoglobin from a solution of it in serum when placed in contact with it for a minute or two?

I used another method, that of Hoppe-Seyler, to determine the serum n the sediments in order to have a double check. In a number of the xperiments both methods were applied to the same sediment. The greement in the results was very satisfactory. This was considered to ntitle the data to greater confidence. According to Brooks, this was a omplete delusion. For "*if*" in the washing of the sediment, entailed in Ioppe-Seyler's process, hemoglobin (and proteins) came out of the coruscles the volume of corpuscles would be underestimated. It had to be nderestimated so as to account for the discrepancy with the hematocrit esults without admitting that the hematocrit usually gives sediment olumes which are rather too high. *If* I had employed a third method it iight have been somewhat puzzling to know which direction the hemolobin ought to take, but I have confidence that Brooks would have found way. As in the case of the colorimetric determination, he made no bservations to prove whether a sufficient loss of material from the erythro-tes to influence the results sensibly could actually occur. He italicizes e word "wash" in speaking of Hoppe-Seyler's method, as if that was a proach. Yet washing erythrocytes is a well-understood and extremely mmon process. All the serologists in the world use it. I have washed ythrocytes hundreds of times without seeing anything remotely resem-ing the disturbing picture conjured up in the "critical examination." I mit that erythrocytes can be badly washed, but persons who do not ıow the proper technique ought not to be washing erythrocytes; they uld be harmlessly employed as critics.

Brooks' analysis of one of my experiments in which the serum was termined in duplicate by a colorimetric method and also in duplicate by ppe-Seyler's method illustrates the thoroughness of his criticism. He es not mention the important point that the duplicate colorimetric terminations gave 58.843 cc. serum and 59.711 cc. respectively in 100 cc. od (Δ = 1.47 per cent) while the duplicate Hoppe-Seyler determinations ve 59.051 cc. serum and 58.480 cc. respectively (Δ = 1.03 per cent). ean of the duplicates by colorimetric method 59.277; by Hoppe-Seyler's

method 58.765 (Δ = 1.15 per cent). It should be remembered that in the colorimetric determination over 93 per cent of the serum by volume was directly measured by centrifugation; it was only less than 7 per cent of residual serum which was estimated colorimetrically. There was obviously no chance for any serious error here. The Hoppe-Seyler estimation, which agrees within about 1 per cent with the colorimetric estimation is also clearly an accurate determination; otherwise there could be no such agreement. The mean of the Hoppe-Seyler and colorimetric estimations is 59.02 cc. of serum; the electrical method gave 59.39 by one formula and 59.73 by another (mean 59.56, or only 0.9 per cent more than the mean of the colorimetric and Hoppe-Seyler determinations). Is it not clear that such an agreement between the results of these three methods could only be attained if all three were right? Is it not ridiculous for a critic, who professes to analyze this very experiment, to sit down at his desk and prove that if a certain amount of the protein and hemoglobin of the cells was lost in washing, the amount of serum should have been more than 10 cc. less in 100 cc. of blood than I found it? What the experiment really proves is that if any loss of protein and hemoglobin occurred it was so insignificant that it produced no sensible effect on the serum estimation. A ten per cent error would have been revealed by a gross disagreement with the other methods. It is surely childish to insist that if an error was committed, which the experiment shows could not have been committed, the serum would have been wrongly estimated by over 10 per cent, whereas the data prove that it was correctly estimated.

But let it be admitted for a moment that the erythrocytes in the sediment were underestimated by both methods, how does it happen that the degree of underestimation is the same, so that an excellent agreement in the results of the two methods is obtained? How can the quantity of hemoglobin taken up by the erythrocytes in the colorimetric estimation be such as to give the same underestimation of the erythrocyte volume as the quantity lost in washing in Hoppe-Seyler's method? There is no connection between the two; it is quite impossible that in a number of experiments these quantities should accidentally agree. The true explanation of the agreement of the results of the two methods of estimation is that they both measure the proportion of serum and erythrocytes with satisfactory accuracy.

Again, it is impossible to see, if the discrepancy between the results of the hematocrit and the electrical method are due to underestimation of the erythrocyte volume in the sediments, how the data obtained by the electrical method should usually come so near those obtained by the hematocrit and should never exceed the hematocrit values, being nearly always a little less, as they ought to be. It is absurd to suggest that this could be due to any approximately constant, small underestimation by two totally

fferent methods, hemoglobin migrating into the erythrocytes in the one se, and out of them in the other in the proper proportions to produce just e difference of 2 or 3 cc. or less of erythrocytes in the 100 cc. of blood tween the hematocrit and the electrical determinations.

It might have been supposed that the close agreement of the values lculated from Fricke's formula with my experimental data would have en a point in favor of the accuracy of the latter. But Brooks does not ink so, and he concludes that the agreement does not help the experimen- data at all. They remain just as "unsound" as they were, perhaps more The formula, on the other hand, which agrees with the experimental ta, emerges without a stain on its character, after certain cosmetic erations by Brooks. My own opinion is that neither Fricke's formula r my data can either gain or lose by this kind of critical examination, ich however, does get pretty tedious.

ONDUCTIVITY OF INTERIOR OF ERYTHROCYTES. Many years ago Höber empted to estimate the conductivity of the interior of erythrocytes. cke's more recent work has advanced our knowledge of that difficult tter. He finds that the conductivity is about one-third that of serum. it is assumed that hemoglobin is present in the erythrocytes to the ount of about 30 grams in 100 cc. of the moist corpuscles, and that it is such a condition as to depress the conductivity of the erythrocyte con- ts in the same proportion as it would that of serum (Stewart, 1899a), s factor alone would account for a reduction of the conductivity of intracorpuscular liquid from a value equal to that of serum to a ue less than half that of serum. It is therefore quite possible that the racorpuscular liquid if taken separately from the hemoglobin might e a specific conductivity not much inferior to that of serum.

et K (25°) $\times$ 10^4 = 150 for serum after eliminating the depression to proteins. Assume this value for the protein-free and hemoglobin- intracorpuscular liquid. Deducting the diminution of conductivity sed by 8 per cent of proteins and, say, 32 per cent of hemoglobin in 100 of intracorpuscular liquid, we get about 65 as the value of K (25°) $\times$ for the interior of the corpuscles, i.e., between one-half and one-third value for serum. A result which seems to follow clearly from Fricke's mate is that the low specific conductivity of erythrocytes is not due nly to a low conductivity of the interior. The alternative is a poorly ducting outer layer or envelope, and there is good evidence that this is sent in many cells, including erythrocytes.

SUMMARY

he specific conductivity of well centrifuged sediments of blood has been nd to be considerably less than one-hundredth of the conductivity of the m ($\frac{1}{103.6}$, $\frac{1}{111}$, $\frac{1}{123}$, $\frac{1}{127.3}$ in 4 specimens of blood). In two specimens

it was $\frac{1}{80}$ and $\frac{1}{88.6}$ respectively; and values ranging from $\frac{1}{30}$ to $\frac{1}{42}$ were found in 4 specimens. There is reason to believe that still lower values than the lowest measured can be obtained for sediments; and it is certain that the larger conductivites could have been lowered by better choice of the speed and duration of centrifugation. It must be remembered that even in the best separated sediment some serum is present, so that the actual conductivity of the erythrocytes is no doubt much less than that measured in any of the sediments. The presence of 1.3 per cent of serum in the sediment showing the lowest conductivity would account for its conductivity. This proportion of serum would be more than furnished by a layer of serum around each erythrocyte 0.01μ in thickness, which would correspond to about 1.7 per cent.

Inspection of the data or of the graphs plotted from them, an example of which is given in figure 1, shows that if extrapolated beyond the point corresponding to 90 per cent of serum the curve will cut the ordinate representing a ratio of $\frac{k_1}{k} = 1$, corresponding to 100 per cent of serum' approximately at the 100 per cent line. This must be so if the experimental data from which the graphs are plotted are correct, as there is no doubt they are. At the other end the curve approaches the abscissa axis asymptotically as $\frac{k_1}{k}$ increases. The maximum value which can be actually measured for $\frac{k_1}{k}$ has not been determined. The estimation of the conductivity of erythrocytes alone is complicated by the existence of some serum in the sediment, after the most thorough centrifugation. If $\frac{k_1}{k}$ reaches a maximum which cannot be exceeded, the corresponding point on the curve will be slightly above the abscissa axis.

Beyond a certain point continued centrifugation may be associated with a great increase in $\frac{k_1}{k}$ compared with that found in the same blood at an earlier stage. When some hemolysis occurs in prolonged centrifugation, the serum, previously free from hemolgobin, becoming tinged, the ratio of the conductivity of the serum to that of the sediment increases. This is due to an increase in the conductivity of the sediment, with practically no change in that of the supernatant serum. The viscosity of the sediment diminishes at the same time.

The addition of a trace of saponin in substances to a sediment greatly increases the conductivity (to 20 times or more) and diminishes the viscosity.

My previous conclusion that the hematocrit yields somewhat too high values for the sediment is confirmed. Reasons are given for this.

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THE ADRENAL GLANDS *

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Since 1915, Dr. J. M. Rogoff has collaborated with me in all the work on the adrenal glands. It has been a joint research, and even when I speak in the first person singular I include him. Further, the investigation, although extending over a long time, was planned as one continuous piece of work on the two glands constituting the adrenal bodies. As it was planned, so it was carried out, with few modifications. The work on the interrenal gland overlapped that on the chromaffin tissue, but when the latter had been proved to possess only slight physiologic significance, or, at any rate, not to be concerned essentially in the preservation of health and life, we decided to spend no further time on the medulla but to concentrate all our efforts on the cortex. Many interesting facts, however, were discovered in regard to the liberation of epinephrine from the medulla, and previously known facts were confirmed and amplified, some of which are herein mentioned.

In this paper, no attempt is made to cover the whole field of the physiology and pathology of the adrenal glands. This is much too extensive even to be sketched in the time at our disposal. The literature is vast and continually growing. Unfortunately, much of the work is, for various reasons, so poor that it is kept alive only through the conscientious efforts of authors to notice everything that has been written. It would be a distinct advantage if many papers, not necessarily the earlier ones alone, could be forgotten. To describe everything in detail, to analyze and to controvert what is wrong would require the writing of a shelf of books. A complete account even of what has been already ascertained, or of what is more or less plausibly conjectured, would fill a good sized volume. All that can be done here is to select what seem the most important and best established results, particularly the more recent ones, to connect them, as far as possible, with each other and, perhaps, in certain instances to relate them to properties and functions of other tissues, as a contribution toward a future theory of the functions of the glands.

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The active substance of the medulla having been discovered and isolated at a time when hardly anything was known about the cortex, it is unfortunate, though natural, that adrenalin or epinephrine should have at once assumed the place of the hormone of the adrenal gland. It has been so designated almost everywhere, not only in textbooks of physiology but, with hardly an exception, in textbooks of medicine and in clinical writings generally; this error has persisted to the present hour. As the adrenal gland is unquestionably important, being, in fact, indispensable to life, it seemed necessary to invest the only known active principle of the gland with important physiologic functions, particularly as the pharmacologic actions of the substance (usually in much larger concentration than can ever exist in the arterial blood) are manifold and most striking. Everywhere, then, the importance of epinephrine was emphasized. It sustained the blood pressure by its vasoconstrictor action. It titillated the heart when it tended to lag and grow weary and kept it to its never ending task. It influenced the tone of all muscular structures innervated by the sympathetic nervous system. It caused the pupils to dilate, the eyeballs to appear more prominent and menacing, and the hair on the tail and back to be erected when animals were stirred by rage or anger. It participated in the physical accompaniments of the emotions aroused by combat. It even caused the blood to clot faster if the combatant was wounded. It was the only one of the internal secretions which seemed to be completely anti-pacifist. No wonder it became popular. No wonder many came to agree with Cybulski, who wrote at the time when indications of what is now called epinephrine or adrenalin were found by him in the adrenal veins: "The nervous system is now dethroned." This absurd dictum and all that it implies became embedded in the physiology of the clinician. If standard textbooks of medicine are consulted today as to the pathology of Addison's disease they still announce that it is the loss of epinephrine that causes the chief symptoms, including, of course, the fall of blood pressure. Usually the cortex is never mentioned. The consequence of all this is that the average reader rises from his study of the works usually available to him deeply impressed with the varied and potent actions of the epinephrine liberated by the medulla, but scarcely conscious that the cortex is anything else than what used to be called a vestigial remnant without physiologic significance. It is probably safe to say that nine tenths of the medical profession are completely ignorant of the physiology of the adrenal bodies, or, what is far worse, have minds so well stored with erroneous information that truth finds them hard to penetrate. Many generally well informed students, if asked what would happen were the adrenal medulla to cease delivering epinephrine to the blood, would probably

answer that a severe fall in the blood pressure would occur, while the emotions of the sufferer, in the presence of the physiologic catastrophe, would have to remain unexpressed.

I am not sanguine enough to suppose that such errors can be eradicated easily; nor is it my business to carry on a crusade against them. It is not important that they should linger on for a while. In time all these matters will be put right. On the present occasion, however, the opportunity offered of restating the salient facts in regard to the supposed functions of the medulla need not be neglected. The chief part of the paper will deal with the newer work on the cortex.

To forestall a possible criticism, or at least to prevent ambiguity, it may be stated at the beginning that I generally use the term adrenal rather than suprarenal even in the cases in which the latter is anatomically correct, as in man. In one of the common laboratory animals, the guinea-pig, the large adrenal bodies lie cephalad to the kidneys rather than beside them, and might be denominated suprarenal rather than adrenal glands, if the mere anatomic relations were to govern the terminology. These relations, however, whatever their occasional interest in surgery and pathology, have no functional significance. It seems better, therefore, to employ one name for the same tissues irrespective of their anatomic relations to the kidneys.

The important experimental data have been obtained on the adrenal glands of animals. If the same word is to be employed to characterize one and the same structure, the use of the term adrenal should be extended to take in the human gland. The opposite rule of using the word "suprarenal" indiscriminately in dogs, cats, rabbits, rats, guinea-pigs, etc., as well as in man, is insisted on by some editors for reasons difficult to fathom. It certainly seems clumsy to speak of the suprarenal glands of a rat or a cat—quite as clumsy as it would seem to speak of the adrenal glands of a man. No harm is done in either case if the self-respect of the necessary, harmless contributor is not outraged in the process of securing uniformity.

More than seventy years have elapsed since Addison published his observations on the "constitutional and local effects of disease of the suprarenal capsules." It shows a penetrating judgment that from a clinical and postmortem study of certain cases of the syndrome now called Addison's disease he should have unveiled a relationship, since abundantly confirmed, between the syndrome and a destructive lesion, especially tuberculous, in the suprarenal glands. His observations, however, are not always easy to understand. For instance, in four of the eleven cases described, the lesion was unilateral. Now destruction of one adrenal gland in animals is harmless. Yet, apparently, the

clinical symptoms were present in these four cases as well as in the remaining seven in which the lesions were bilateral. It may be that Addison was in error in regarding the lesion as unilateral in the four cases mentioned. Microscopic examination might have revealed destructive changes in both capsules. Certain more recent writers, however, have described cases of Addison's disease associated with a unilateral lesion.

At that time it was not recognized that the adrenal body in mammals consists of two glands, the cortex or interrenal gland and the medulla or chromaffin tissue. Even to this day it is not uncommon for authors to refer to the adrenal gland as if it were homogeneous in structure and in function. No apology, therefore, need be made for stating at this point that the cortex and medulla are considered herein as two distinct organs. The mere fact that the medulla is embedded in the cortex changes nothing except that it entails some modification of the blood supply. Until this was made clear, it was impossible to advance far in the study of the adrenal glands, just as it was impossible to advance far in the study of the thyroid gland until the parathyroid glands were discovered and shown to be separate organs with completely different functions.

Several years ago, in discussing the subject of adrenalectomy and the relation of the adrenals to metabolism, I stated that "if the cortex is the important part of the adrenal it would seem logical in considering the special relations of the gland to metabolism to take the cortex first. It happens, however, that by far the greatest amount of work has been done upon the medulla, the reason being that it forms and gives off a well characterized substance, epinephrine, which in sufficient doses causes many striking reactions, including definite metabolic effects." For that reason the medulla was taken up first and at considerable length, while the more important cortex was relegated to the end of the paper and treated briefly. In the interval which has elapsed, much progress has been made in knowledge of the cortex, largely through the researches carried out in this laboratory by Dr. J. M. Rogoff and myself. Dr. de Necker, Dr. R. Dominguez and Dr. Ecker have also collaborated with Dr. Rogoff in investigating certain points. It will, therefore, be best to reverse the order in the present paper and to deal first with the really important gland of the adrenal body, the interrenal gland or cortex.

THE INTERRENAL GLAND OR CORTEX

It is not possible to extirpate the cortex except by removing the entire adrenal body. Proof will be given that the medulla or chromaffin tissue can be taken away without affecting the health or life of the

animal. The consequences which follow total adrenalectomy are quite different and may, therefore, be attributed to loss of the function of the cortex.

A large proportion of white rats survive indefinitely the loss of both adrenal glands. The same is true of wild (sewer) rats, as shown by Boinet more than thirty years ago. Rogoff and de Necker found that in large batches of good stock, fully 50 per cent of white rats, and in some batches still more, may survive, apparently for an indefinite time. Of adult rabbits, not less than 25 per cent—under the best conditions, considerably more—recover completely after excision of the two adrenal glands and live indefinitely. A large additional number survive for several weeks. The fact that many rats and rabbits survive the loss of the adrenal glands has generally been attributed to the presence of accessory adrenal glands composed of tissue like the cortex, so that in these animals the operation sometimes remains incomplete. While this explanation may be accepted for some of the animals it cannot be considered as proved for all. Nor has the amount of tissue necessary to keep an animal alive been ascertained so exactly that an accessory, when found can be confidently said to suffice.

The dog is an animal in which, according to macroscopic observations, accessory adrenal glands are exceedingly rare. One was encountered in 150 dogs, and this was seen at the time of operation and removed. Dogs always die after total adrenalectomy. If the technic is adequate, they do not die from the operation as such but from the syndrome which is called adrenal insufficiency, and which develops after a longer or shorter period of good health. We have accumulated no less than 200 of these "control" dogs in which the adrenal glands were removed one at a time, with an interval of from one to many weeks between the two operations. The results on the control animals, not subjected to any treatment, form the indispensable standard of comparison when a given method of treatment, or the effect of certain physiologic conditions on the consequences of total adrenalectomy is being tested. In certain cases, the control series has been swelled by the addition of animals in which a given treatment has had a clearly negative result. It is not necessary to reproduce tables in which the results on control dogs have been embodied. They can be found in several of our published papers.

It may be said that most dogs operated on according to our technic survive the removal of the second adrenal gland for at least a week. Many live for ten or twelve days, a few for fourteen or fifteen days, and in rare instances the dogs live for fewer than five or six days. One dog of 150 was seen to survive sixteen and a quarter days. None of the dogs die in as short a time as twenty-four hours, and we are inclined to believe that when an animal survives for only two or three days, it does not die of adrenal insufficiency alone. We do not think

averages of such series of much use, but the average for our dogs is about eight days; for the last series it was nine and a half days.

Cats survive longer than dogs. The average in our laboratory for nearly fifty cats was eleven days. The majority lived for from one to two weeks. A few survived three weeks, and one about thirty days. One cat survived for thirty-one and a half days, and one, a castrated male, which was not included among the controls, more than thirty-five days. In accumulating control dogs and cats, only male or nonpregnant female animals are chosen. No female ascertained to be in heat at the time that the second operation would be performed is taken. The reasons for these restrictions will appear later.

It scarcely need be said that in all animals used in either series, for control or treatment, most careful search was made both at operation and post mortem for accessory adrenal glands. Accessory adrenal glands were seen in two cats and removed at operation. In one cat, two accessories were observed at the first operation and purposely left behind; a third was found post mortem. The animal lived for fourteen days and was not included among the controls.

Guinea-pigs in our laboratory have been found to survive much longer than any mentioned in the literature, although not as long as cats or dogs. Many of them live about a week—a few longer.

We have dwelt on this matter of the control series because in judging the effect of a given treatment or a given physiologic condition on survival, everything depends on a comparison with a sufficiently large series of controls. In our opinion, these should be operated on by the same observers who operate on the animals in the treatment series and under the same conditions. Only when both series are accumulated in the same laboratory can a real check be made.

Our results differ greatly from most of those in the literature. Beginning with Brown-Séquard, and in the case of other animals than dogs, statements as to the time of survival and the behavior after adrenalectomy are seen to be impossible of acceptance, because the animals obviously died as a result of poor surgical technic rather than adrenal insufficiency. The symptoms of adrenal insufficiency could rarely, if ever, develop clearly and uncomplicated by other symptoms not connected directly with elimination of the cortical functions. Whatever study might be given to the picture, it was bound to be unsatisfactory because of the clouding effects of the operation. Further, the study was necessarily too transitory because the period of survival was so short.

Perhaps the best illustration which can be given of the absolute necessity of good technic in drawing conclusions as to the effects of loss of the adrenal glands is the work of Brown-Séquard. Not long after the publication of Addison's paper, he extirpated the glands in

different species of animals, and is credited with being the first to prove that they are indispensable to life. This conclusion is certainly true in dogs, cats and guinea-pigs, in most rabbits and in some rats; Brown-Séquard, however, did not prove it. All the animals which he operated on, including rats, rabbits, guinea-pigs, cats and dogs died in from nine to thirty-seven hours. Even when he removed only one gland from rabbits, guinea-pigs, cats and dogs, none survived longer than from twenty-three to twenty-four hours. It is now well known that removal of one suprarenal gland, far from being fatal, does not affect the health or shorten the life of any animal operated on under proper conditions. It was pointed out at the time by Phillipeau that there were other causes than the loss of an indispensable function which might account for the fatal consequences of Brown-Séquard's operations, for example, hemorrhage and sepsis, both extremely common. Cooling, perhaps undue prolongation of operations, inadequate care before and after operation and excessive trauma in a field peculiarly susceptible to trauma almost certainly contributed to the bad results. In any case, what has been said shows clearly that Brown-Séquard was not in a position to draw any conclusion from his results.

It has not been sufficiently recognized, perhaps, that the surgical difficulties in adrenalectomy are, except in rats, greater than in operations on most of the other endocrine glands. Removal of the thyroid gland is simple; so is removal of the parathyroid glands as soon as they are recognized. Gonadectomy is easy in the male and not difficult in the female in the laboratory animals. Complete pancreatectomy is, of course, an operation requiring skill. Operations on the pituitary gland are difficult on account of the formidable approach and for other reasons. In the case of the adrenal glands, the right gland in the guinea-pig lies close against the vena cava and under the liver; the vessels are delicate and liable to injury. In the rabbit this is true also, but not to the same degree. In the dog, the operative field lies deep and the right gland is close to the vena cava. Next to the guinea-pig, then, among the common laboratory animals, the dog is the animal whose right adrenal gland is most difficult to excise. In the cat, the operation presents no special difficulty on either side. Except for the rat, this is the animal in which the operation is easiest. In all the animals, adrenalectomy is simpler on the left side than on the right.

We believe that not nearly enough attention has been given to the proximity of great numbers of sympathetic fibers and ganglions which are liable to be crushed and torn unless the operator knows the importance of inflicting on them as little injury as possible. The removal of the adrenal glands or of one of them, unless it is done with care, is apt to cause a fall of blood pressure which has nothing to do with the loss of epinephrine previously discharged from the gland; it is,

instead, the expression of a shock-like condition apparently due to the traumatization of these numerous sympathetic fibers. We have often seen this in acute experiments in which the adrenal glands were removed to test the effect on some reaction which was previously obtained. When this shock-like condition supervened, such a reaction as acceleration of the "denervated" heart or vasoconstriction of a denervated limb on stimulation of sensory nerves might disappear, since shock was, of course, unfavorable to eliciting the reflexes. If the blood pressure later recovered with the adrenal glands removed, the reaction might again be obtained. It had not been suppressed because of loss of the epinephrine but on account of the shock caused by the trauma. In view of the fact that no other factor has been responsible for errors in work on the adrenal glands to the same extent as inadequate surgical measures, no apology is required for spending some time on the matter.

SYMPTOMS FOLLOWING ADRENALECTOMY

We return to a consideration of the consequences of complete adrenalectomy, which can be studied perhaps most satisfactorily on the dog. A short time is required for a dog to recover from the anesthetic. One of the advantages, probably not a slight one, of extensive experience in performing the operation is that the duration of the anesthesia can be diminished. The ordinary time of the operation with us is from eight to twelve minutes in a dog, and about five minutes in a cat. By that time, the skin is stitched and the anesthetic discontinued. It is, therefore, not to be wondered at that in a half an hour, sometimes less, the dog has generally recovered and is willing to eat, though not permitted to do so. The period of good health is proportional to the total period of survival, so that whether the latter is long or short, about the same number of days elapses between the first development of serious symptoms and death. During the period of good health the animal appears perfectly normal. It preserves habits or tricks which were characteristic before the removal of the second adrenal gland. It is impossible to determine from the animal's behavior whether it has lost its adrenal glands or not. Appetite is unaltered. Dogs that ate voraciously before loss of the adrenal glands eat voraciously after the glands have been excised; those which ate slowly continue to eat slowly. Dogs that liked meat still do so, and dogs previously fond of bread and milk continue to like this ration. The blood shows no changes so far as they have been looked for. The specific gravity, the red cell count, the hemoglobin percentage, the conductivity of the blood and serum, the relative volume of corpuscles as compared with serum, the nonprotein nitrogen, the urea nitrogen, the so-called "undetermined fraction" of the nonprotein nitrogen, the uric acid, the creatinine and the amino-acid nitrogen are not altered, except that in

some cases an increase in the nonprotein nitrogen, urea nitrogen, "undetermined fraction" and relative volume of erythrocytes may be detected a day or so before the first definite refusal of food.

Anorexia is the first and most constant of the serious symptoms which terminate the period of good health. Once declared, it persists, except that occasionally an animal after finally refusing the usual ration may be tempted to eat once, rarely more often, something which it considers a delicacy. Refusal of food is, of course, a common symptom in any sick animal. It cannot be said that there is anything especially characteristic about the anorexia of adrenal insufficiency, except that it is inexorable and that it presages the death of the animal in, at the most, a few days, usually two or three. To an observer who does not know that the adrenal glands have been removed, there is nothing which distinguishes this anorexia from any other anorexia, unless it is an aversion to fat which develops before total anorexia has set in. This point will be considered in connection with the changes in the pancreas, and again in connection with the aversion to fat seen in cases of Addison's disease. The anorexia may be accompanied by emesis. Bile may be present in the vomitus. As a rule the animal does not appear seriously ill at this stage. It is constantly a source of surprise to the observer until he gets accustomed to it, how slight an ailment adrenal insufficiency usually appears to be in a dog, at least until near death. It is the uniform and rapidly fatal result which shows that the symptoms, whether slight or not, are indications of a serious disturbance.

Asthenia, which has been emphasised by some authors as the chief and earliest symptom, is not as a rule obvious for some time after the animal has definitely refused food. The error is due to the fact that moribund animals were observed, dying mainly as a result of the operation. Naturally, dying animals are weak. Often the animals are strong on their feet, pugnacious if they were so before, and able to run well if they can be coaxed to try. They are, however, apt to be lethargic; as time goes on, the lethargy deepens into stupor and coma. Convulsions are sometimes seen. On the whole, however, the behavior of the animal after anorexia and the other symptoms have developed often by no means suggests danger to an inexperienced observer. The arterial blood pressure (systolic and diastolic) is not affected by the removal of the adrenal glands, as shown in dogs and rabbits by Rogoff and Dominguez by means of the carotid loop method of van Leersum. The pressure was taken at frequent intervals beginning before the removal of the first adrenal gland. Even during the operation, pressure readings were made, as stated, without revealing any change beyond the limits of the ordinary variations. The fall of blood pressure so commonly seen in Addison's disease is, therefore, not reproduced in experimental

adrenal insufficiency, probably because the latter condition is not sufficiently chronic. Undoubtedly, when the animal's circulation has become impaired, as commonly happens when death approaches, there is a fall in blood pressure. It has often been remarked that the pigmentary changes so characteristic of Addison's disease are not found in experimental adrenal insufficiency. One could not expect to find them in animals living only a few hours or days, or, on the other hand, in animals living on indefinitely because of the retention of sufficient interrenal tissue to maintain life. The picture seen in dogs deprived of their adrenal glands is, accordingly, not quite the same as that seen in diseases of human beings. It must be remembered that in Addison's disease the loss of the interrenal tissue is usually gradual as the pathologic process spreads. Sometimes it is apparently due to atrophy or, perhaps to congenital deficiency of the tissue, the glands being represented at necropsy by a few small nodules of the same structure as the cortex.

CAUSE OF THE SYMPTOMS AND DEATH

When the question arises as to what causes the symptoms and death in experimental adrenal insufficiency, it must be confessed that at present no definite reply is possible. It must be assumed that an intoxication develops, perhaps creeping on from the first day although not announced for some time by detectable symptoms. The remedial effect of large intravenous injections of salt solutions (Ringer's solution with dextrose) is scarcely to be explained except on the supposition that an intoxication has been produced, through the lack of a substance or substances elaborated by the cortex which prevents such intoxication, or of something which aids in neutralizing the toxic products and rendering them harmless. The increase in the nonprotein and urea nitrogen of the blood and in the "undetermined fraction" of the nonprotein nitrogen, and the diminution in the ratio of plasma to corpuscles, however related to the loss of interrenal function, advance in equal measure with the terminal symptoms. They may, as already mentioned, overlap by a day or so the latter part of the period of good health. The concentration of the blood sometimes becomes extreme, as many as 10,000,000 erythrocytes to the cubic millimeter being present, with the specific gravity of the blood exceeding 1.070. The concentration does not affect the serum, its specific gravity rarely exceeding 1.025 and its protein content being unaltered. The serum calcium usually undergoes an increase at the time of, or sometimes a little before, the development of the serious symptoms, especially anorexia, which terminate the period of good health. The blood sugar remains within the normal limits till near death, when a moderate diminution may occur. The uric acid of the blood undergoes no important change. The creatinine and amino-acid nitrogen sometimes show a small increase. The blood chlorine often undergoes a moderate diminution.

If metabolic or other derangements begin immediately or soon after the removal of the second adrenal gland, they have not yet been discovered. It may be that defensive, neutralizing mechanisms are at work which, for a while, are capable of dealing with the poisons, but ultimately break down. Later on, evidence will be given that the interrenal tissue contains a substance which, when administered to adrenalectomized dogs can lengthen the period of survival beyond anything seen in the control series. If this substance is a hormone normally given off by the gland, it may act by influencing the function of some of the important organs which fail sooner or later in its absence, or it may influence special metabolic processes. Then these organs or processes will be interfered with, and in addition poisons may be produced. Thus, in the case of the internal secretion of the pancreas, insulin supplies something necessary for the normal metabolism of carbohydrates, but in its absence toxic substances are also produced which are responsible for such symptoms as coma. The administration of insulin prevents the development of these poisons by restoring normal metabolism.

In one endocrine organ after another—thyroid and parathyroid glands, pancreas (islets) and interstitial tissue of gonads—active substances have been discovered which substitute for the glands. Even the adrenal medulla, the chromaffin tissue, perpares and discharges into the blood a specific substance, epinephrine which, although of slight physiologic significance, belongs to the group of hormones. Analogy suggests that the adrenal cortex or interrenal gland prepares and liberates a hormone also. To this substance the name interrenalin is given, although it has not yet been isolated.

The question at once occurs: Why do some dogs live much longer than others? No definite answer can be given, but one or two suggestions may be made. It is possible that some dogs have a larger store of the active substance than others, if the substance is stored in other tissues than the interrenal glands. This is on the assumption that it is manufactured solely in the adrenal glands. If so, it may be taken up and stored in other tissues, possibly in the interstitial cells of the gonads or, in the case of the female, in the elements from which the corpus luteum is developed. If that is the case, some dogs may store more than others and, therefore, have more at their disposal when the second adrenal gland is taken out. Then these tissues, the origin of which is probably similar to that of the interrenal tissues, may normally elaborate interrenalin, although it may not be liberated in the absence of any need for it. If liberated it would naturally constitute a small contribution compared with that of the adrenal glands. Another possibility is that some dogs may use up any store of the active substance with greater economy than other dogs. Finally, there may be no store whatever, but the resistance of some dogs to the consequences of loss of the adrenal

glands may be greater than that of others, just as the power of resistance to a disease or a poison may vary. None of these suggestions, or others which might be made, have been tested. The fact, however, that a range of from three to five days to fourteen days or more exists in the period of survival of dogs which possess no interrenal tissue invites speculation. Could the meaning of this variation be understood, much light would probably be thrown on the general problem of interrenal function.

POSTMORTEM OBSERVATIONS

The postmortem observations on dogs dying of adrenal insufficiency present some points of interest. The most constant change observed is congestion of the pancreas, usually intense and seldom absent. In cats and guinea-pigs, this is also a practically constant feature, although, perhaps from the small size of the organ, the pancreas is not so red. In all these animals, we have compared the postmortem observations with those in normal animals, and the difference is marked. The meaning of the congestion is unknown, but it would suggest some involvement of the organ due to the loss of the interrenal function. It cannot be stated at present how early this congestion is present; it is possible that it is only a terminal event, developing, perhaps, at about the same time as the anorexia or even later. In animals that have been examined microscopically, it appears to involve both acinar tissue and islets. To what extent, if at all, the secretion of pancreatic juice is affected by the changes in the pancreas, it is at present impossible to say. The loss of appetite for fats at a time when lean meat is still picked out and eaten may be connected with an impairment of the fat-digesting power of the pancreatic juice. In cases of well marked hemorrhagic congestion, the condition of the mucosa of the intestine would itself interfere with the digestion and absorption not only of fats but of other foods. Another striking appearance, although not so constant, is hemorrhagic congestion of the mucosa often associated with the presence of blood in the lumen of the gastro-intestinal tract. Sometimes intense congestion, with hemorrhage into the mucosa and blood in the lumen, is seen throughout the whole gastro-intestinal tract from the cardiac end of the stomach to the anus, but often only a portion or scattered areas of the tract are affected. Bile is nearly always found in the stomach. The esophagus never exhibits any congestion. It is occasionally present in the vermiform process and often in the the cecum. In exceptional cases, there is neither congestion of the mucosa nor blood in the lumen. It is probable that the gastro-intestinal congestion is a rather late event, but there is no exact information on this point. It may be present, however, some days before death; blood is sometimes passed from the rectum. The significance of these pathologic changes in dogs dying

of adrenal insufficiency is unknown; only they are often so extensive and severe as of themselves to constitute a sufficient cause for the fatal result, and, of course, if present early enough, for the onset of the characteristic anorexia. The congestion in the gastro-intestinal tract and in the pancreas is not merely a part of a general passive congestion of the viscera due to failure of the heart, for it may be extremely marked when the spleen, kidneys and other organs are only moderately or not at all congested. We do not desire to emphasize unduly the gastro-intestinal appearances, for it is known that in other conditions somewhat similar appearances may occur. In cats dying after adrenalectomy these changes, although often seen, are not usually so intense.

We have suggested the possibility that the gastro-intestinal mucosa may share in the elimination of the poison or poisons developed in adrenal insufficiency and may itself be finally crippled by the poison. An alternative hypothesis is that the cortex produces a hormone necessary, among other actions, for the continued normal functioning of the mucosa and also of the pancreas. In the dog, the appearances suggest that a disturbance of function centering around the alimentary canal is one of the consequences of adrenal insufficiency. That there should be no hemorrhage or hemorrhagic congestion in some of the dogs is, of course, no proof that the gastro-intestinal tract continues to function normally. The persistent anorexia and not uncommon emesis may be primarily due to gastro-intestinal changes not necessarily evidenced by congestion. The gastro-intestinal mucosa may be just as much a point of attack in the cat and guinea-pig, and in those dogs which do not show the appearances in question, as when the striking hemorrhagic changes are present. It is not worth while discussing at present whether the smaller dimensions of the structures, possibly even differences in the quantity or quality of the residues of food in the gastro-intestinal tract, may have a bearing on the matter.

THE CAUSES OF THE BREAKDOWN

It has been said that during the period of good health the adrenalectomized dog seems normal in every respect. Nothing suggests that the condition of the animal is serious and, indeed, hopeless. Is this appearance of perfect health fallacious? Are changes already in train which will soon issue in unmistakable symptoms? These questions cannot be avoided, but neither can they be answered at present. As already stated, search has been made by us for changes in the blood which might indicate that something was amiss, perhaps soon after removal of the second adrenal gland, although no visible symptoms had yet developed. Hitherto, all the results have been negative. Only when the train of symptoms appears which indicates that the period of good health is over do recognizable changes in the blood develop; these usually

increase until death. It is clear enough, however, that none of the changes in the blood studied by us follow soon after the loss of the interrenal function. If a poison begins to accumulate from the outset, although causing no symptoms until it has reached a certain concentration, requiring maybe a week or more, its detection is not likely to be easy. All we can say at present, therefore, is that during the period of good health the animal runs along on whatever reserves of interrenalin it possesses, if it possesses such reserves till in a week, more or less, the derangement of function leads to symptoms. If poisonous substances, produced in the absence of the interrenal tissue, are being eliminated or neutralized, it is possible that the period of good health is terminated by the breakdown of the organs concerned, perhaps owing to the harmful effects of the poisons on their own cells. At one time, it was suggested by certain writers including the author that the kidney might possibly be such an organ.

While no exact study of the kidney has been made, such observations as we have accumulated do not indicate that in the dog the kidney is specially concerned. The occasional pathologic changes seen in one or both kidneys are probably no greater than would occur in any large series of normal dogs. In rabbits, interference with a kidney at operation may account for some of the cases of renal change. We are far from regarding the pathologic appearances in the dogs' gastro-intestinal tract as proof that the mucosa takes a leading part in the elimination or neutralization of poisons produced in the body in the absence of the interrenal tissue. Nevertheless, it is well known that the mucosa can take an important place as an excretory organ under certain conditions; for example, when saline solutions are injected into a vein. It was shown by Sherrington long ago that much of the salt solution passed rapidly into the lumen of the intestine and was then gradually reabsorbed into the blood and eliminated by the kidneys. We have seen the same thing in experiments in which Ringer's solution was administered intravenously to adrenalectomized dogs. These experiments were undertaken to test the value of such injections as a therapeutic measure in adrenal insufficiency.

METHODS OF TREATMENT

As soon as we had accumulated a sufficiently large series of control dogs, it became possible to test methods of treatment. The only test which at present can be considered unequivocally positive is a prolongation of life decidedly beyond the maximal time seen in the control series. This is a severe test, as it involves the placing of the animal in such a position, through the treatment, that it can successfully meet not only one but all the emergencies which threaten its life. When positive, the test is for this very reason not open to doubt. It

would be much simpler to test the effect of a treatment on a characteristic symptom or on the concentration of a blood constituent, but we are not yet in a position to rely on such tests. Effects such as amelioration of symptoms also have positive value, even when temporary. Let it be repeated, however, that in drawing conclusions as to the efficacy of a treatment we have up to the present refused to accept as a positive result anything short of a decided prolongation of the maximal period of survival, and this in a large proportion of the cases. Some weight can also be given to the fact that a relatively large number of long periods of survival occurs in the treated series, though not necessarily beyond the maximum of the control series. Averages are of little use because of the large variations in the period of survival. They are quite misleading, of course, when they are derived from results obtained in only a small number of animals. A positive result of a given treatment must not be inferred from an apparent increase in the average time of survival unless the first test is positive, that is, unless a considerable number of the animals have lived well beyond the longest period of survival of the control animals.

Injections of Salt Solution.—As stated, injections of salt solution were one method of treatment studied. This was used for several reasons. If an intoxication develops at the time that the serious symptoms appear or is, perhaps, developing from the time of removal of the second adrenal gland, an irrigation of the poisoned tissues, including the blood, which would wash out the whole or a portion of the poison, is possible. A positive result would be that much evidence in favor of the existence of an intoxication; in addition, it would indicate that the poison, or an important fraction of it, was not yet firmly fixed. A salt solution was purposely chosen because its action would be understood to some extent and would be less complex than that of extracts of the glands. There could obviously be no substitution for the missing interrenal gland; nothing in the nature of a hormone could be supplied. Some dextrose was added, but it is not certain that this was of any importance. The intravenous injection of a salt solution is a feasible therapeutic measure; it was thought that if a certain amount of success was obtained in dogs it might well be considered, that in certain cases of Addison's disease, when the condition suggested intoxication and was in any case desperate, an occasional injection of Ringer's solution (in smaller proportional amount than for dogs) might not be justifiable and even promising. It was found that severe symptoms, such as coma and convulsions, could be temporarily ameliorated, and that in some cases animals might be rescued when moribund, and survive much beyond the maximal period of the control series. Further, it was shown that with daily injections of about 100 cc. of Ringer's solution per kilogram of body weight with some dextrose added, many dogs continued in good

health far longer than the maximal period among the control animals. The longest period of survival of dogs treated with Ringer's solution was 53⅔ days, nearly four times as long as the maximum period among the untreated control animals, and not much less than the average gestation period of dogs. Other survival periods among the seventeen treated dogs were 38, 33¼, 32⅔, 20 (two dogs), 19⅓, 19 and 17¼ days. Nine dogs (more than half of the total number) lived beyond the maximal period of the controls, and four additional dogs almost as long as the maximal period.

Nothing like these results was seen among the much larger number of controls. The results of the injections, therefore, were extremely good. It may be pointed out again that there are necessarily great difficulties to be overcome in adding perhaps forty days to the life of an adrenalectomized dog, which without the treatment would usually live only from six to ten days. It is not too much to say that the animal, although running along week after week as if the suprarenal glands were superfluous, is in reality in constant, and, after the ordinary survival period has passed, in imminent, danger of death. Nothing keeps it alive and, to all appearance, in normal health, except the daily liter, or whatever it may be, of a simple solution of salts, such as Ringer's solution. It is not even ascertained that the injection must be a daily one. Possibly less frequent injections would be better. The dose might also have been too large. It was impossible, even though a great deal of time was consumed in the investigation, to compare different doses, different salt solutions, different intervals between successive injections and different periods after the removal of the second adrenal gland for commencing the injections. There is no reason to suppose that we accidentally hit on optimal conditions. Our results are not the best which can be obtained. The unequivocally positive result of the test for lengthening the period of survival is sufficient proof of the beneficial effect of the treatment when carried on for long periods. Even more impressive although not susceptible of the same arithmetical comparison, is the immediate, almost startling, improvement often produced in animals the condition of which seems desperate, for example, in coma. This improvement may not last long in some cases, but in others the dog may take on a new lease of life. Obviously, the result must depend on the extent of deterioration. In one dog, for instance, convulsions and deep coma developed on the third day after the second operation. It was not expected to live through the night. It recovered, however, under treatment with salt solution, regained health and appetite and died on the thirty-fourth day. Symptoms such as anorexia, therefore, may sometimes be benefited, the animal picking up and beginning to eat again. But when the anorexia is absolute and has lasted for some time, this effect is probably rare.

The reason for starting the injections on the day after the second adrenalectomy, that is, long before symptoms of adrenal insufficiency had appeared, was theoretical, although not entirely so. It was thought that, if the injections washed out the poisons, the sooner they were started the better, so as to prevent the toxic substances, as far as possible, from anchoring themselves in vital tissues. Whether that idea is correct or not, it would seem reasonable to suppose that the injections, if beneficial, cannot be begun too early. The postoperative condition of the animal must, of course, be considered. As already stated, our animals recovered from the operation in a short time and were considered fit for injection on the following day. It must be said, however, that a few dogs may not stand so large an injection so soon after a serious operation. It is possible to produce serious effects in such animals, perhaps through dilatation of the heart. The heart's action and pulse should, therefore, be watched while the injection is proceeding. In a typical observation, the dog shows symptoms of increased activity of the bowel during or after an injection, and micturates copiously. If there is no micturition for several hours, suspicion should be aroused.

Administration of Cortical Extracts.—More directly concerned with the cause of death after loss of the adrenal glands and centering around the question of the existence in the cortex of an active substance capable of substituting for it like hormones of other endocrine glands, are our studies on cortical extracts, which have been going on for several years. As a method of treatment in adrenal insufficiency the administration of cortical extracts is, in some respects, the exact opposite of the injection of salt solutions. Yet the results on the lengthening of the period of survival are not very different.

From several of the endocrine organs, specific active substances have been obtained. In some cases it has been proved, in others it is assumed, that the formation of these substances and their liberation into the blood (or lymph) constitute the function of the gland. Assuredly the proof of the existence of these substances, in some instances followed by their isolation and their synthesis (epinephrine and thyroxin) and the determination of their constitution, has not been an easy task. But starting with the work of Baumann, Oswald and others on the active substance of the thyroid gland, followed by the demonstration, by Oliver and Schafer, that a vasoconstrictor substance can be extracted from the adrenal medulla (chromaffin tissue), it has gradually been shown that active principles can be obtained also from the pancreas (islets), from the parathyroid gland and from the sex glands. These have been proved capable of "substituting" for the glands which contain them, in the case of the thyroid gland and the islets of Langerhans, so that in their absence, or when there is undue reduction in amount or in func-

tional capacity, the artificial administration of thyroxin or insulin, respectively, replaces the deficient internal secretion. Important, even indispensable functions are clearly performed by these substances. The specific substance, parathyrin, formed by the parathyroid glands, the loss of which causes tetany, has also been obtained in extracts by Collip. The increase in the serum calcium (hypercalcemia) caused by its administration throws light on the previous discovery of McCallum that calcium salts prevent or cure tetany caused by removal of the parathyroid glands.

It may be mentioned that the largest parathyroid glands that we have ever seen were those of the adrenalectomized dog which lived longest (seventy-eight days) in the series of animals treated with extracts. We do not venture to suggest that the hypercalcemia often seen in these dogs, although only in the terminal stage or slightly preceding it, is brought about through the parathyroid glands. The relations of the adrenal glands, both interrenal and chromaffin tissue, but especially the latter, with other endocrine glands have often been assumed. Proof, however, is still lacking, save in one instance—the correlation of the interrenal gland and certain cells of the gonads (interstitial cells and the corpus luteum?). Since these tissues appear to be derived from the same embryonic structure, the correlation is easy to understand. Changes in the cortex following gonadectomy, and in the gonads following adrenalectomy suggest also the possibility of a functional connection. The best illustration of this is the influence of "heat" (and pregnancy) on the length of life in dogs after loss of the adrenal glands.

Hormones from the ovary and placenta have been described. One, at least, was partially isolated. Replacement effects have been demonstrated in ovariectomized animals, including congestion, swelling and other external signs of estrus (Doisy and other observers).

The adrenal medulla and the posterior lobe of the pituitary gland yield characteristic substances. While of great pharmacologic and therapeutic importance, it is unknown whether they have physiologic importance. It is not even ascertained whether solution of pituitary gives off the active substance to the blood. The question of the function of the epinephrine produced by the chromaffin tissue of the adrenal body will be discussed later in this paper. More or less specific actions, particularly in relation to growth, have been attributed by some observers to extracts prepared from the anterior lobe of the pituitary gland. As investigation proceeded, and as each gap was filled in, there was an increasing probability that the adrenal cortex would in time be added to the list. The discovery was retarded by the experimental difficulties.

No symptom or blood change was known to us which characterized the condition of adrenal insufficiency. We were unable to determine,

as for instance in the case of insulin, by a simple estimation of a blood constituent, the potency of any extract in counteracting the loss of function of the cortex. If this becomes possible, extracts can be tested much more simply than by determining how long animals survive when they are administered. The labor of bringing our experiments to the present point has been great. The work was planned and begun a good many years ago.

The investigations have culminated in the demonstration, for the first time, that cortical extracts can be prepared which can substitute for the adrenal glands. It was indispensable to prove this in order that further attempts to extract and purify the active substance or substances should be placed on a secure basis. The proof that we obtained such extracts is that among the dogs given intravenous injections of certain extracts (generally on alternate days), a considerable number lived far longer than the maximal period seen in untreated control animals. The volume of the injection was usually 1 cc., so that there could have been no irrigation similar to that assumed to result from an injection of Ringer's solution. We are left with the explanation that the extracts supplied something normally supplied by the interrenal tissue. For this we have suggested the name interrenalin.

As in the case of the salt solutions, it is not to be supposed that we attained optimal results with the extracts. Larger and more frequent doses might have been better, but this has not been ascertained. The important point is that we did find extracts and did administer them in doses which were clearly efficacious. The extracts were made from the adrenal glands of dogs or from the cortex of animals from a slaughter house. Probably each batch differed from the rest in the concentration of active substance obtained by extraction. We had no means of standardizing the extracts. Different dogs might have responded differently to similar doses of active substance, some requiring larger doses than others to stave off the fatal breakdown. The possession of a large series of control animals, however, prepared by an adequate technic, enabled us to conclude with certainty that the indispensable test, the lengthening of the period of survival, was strongly positive. The period of good health was correspondingly lengthened. Among the dogs which did not actually live longer than the maximal period for the controls, a larger number, apparently more proportionally than in the control series, attained the longer periods of survival. Occasionally an animal already beginning to go downhill seemed to be revived by a dose of extract, not immediately as in the case of the treatment with Ringer's solution but after some time. The serious symptoms, when they developed eventually in animals treated with extracts, often seemed to be milder than in the controls. The pathologic appearances seen post mortem were also thought to be less pronounced in many

cases. Let it be repeated, however, that at present we do not rely on these subsidiary tests. Our positive conclusion is based on the decided increase in the period of survival beyond the maximal recorded for the control animals. Since the treated animals differed from the untreated controls only in having received extracts, the lengthened survival must have been due to an action exerted by the extracts, which, it is to be supposed, supplied the material (interrenalin) naturally supplied by the cortex.

INFLUENCE OF "HEAT" AND PREGNANCY ON SURVIVAL

The interesting observation has been made by us that dogs in "heat" survive the loss of the adrenal glands much longer than the maximal survival period of the controls. All the dogs from which the second adrenal gland was removed during proestrum or estrus gave this result. One lived until the sixty-fifth day. The period of good health was correspondingly prolonged. The mechanism by which the consequences of total adrenalectomy are staved off must be different both from that of the administration of extracts and from that of the injection of salt solutions. The most plausible view is that changes in the ovary are concerned. The similarity in structure, and in the nature of the inclusions, between the corpus luteum and the adrenal cortex has often been pointed out.

The inclusions in the ovarian interstitial cells also resemble those of the cortical cells. In heat, the changes in the ovary, including the development of the corpus luteum, may render available, in sufficient amount to make up for the loss of the adrenal glands, the indispensable substance produced by the interrenal tissue. As the development associated with heat subsides, this supply must fail and the fatal consequences of adrenal insufficiency then develop. Of course, it is theoretically possible that the ovarian tissues act by destroying poisons produced in the absence of the adrenal glands. Actions of detoxication have been attributed to every endocrine gland until in one case after another a specific substance has been discovered which is necessary to the proper carrying on of particular functions. When that has been done detoxication ceases to be mentioned. It must not be forgotten, however, that when a metabolic process is no longer carried out in the normal manner in the absence of the necessary hormone, the abnormal or incomplete chemical reactions may give rise to the production and accumulation of substances which act as poisons.

Pregnancy, in dogs, is another condition in which we have observed longer periods of survival after adrenalectomy than in the control animals. Naturally in a condition associated in itself with formidable risks, not every animal shows a prolongation of the period of survival. Indeed, not a few succumb earlier than they would have, had they not

been pregnant. Yet it has been clearly demonstrated that many survive from 20 to 30 days, and some from 30 to 40 days. As long a period of survival as 58 days has been seen. One bitch lived for 57½ days. One of the pregnant animals survived for 46 days, one for 32⅔ days, one nearly 27 days and one more than 25 days. Since every pregnant bitch has necessarily been in heat, it cannot be said offhand to what extent pregnancy contributes to lengthening the period of survival. To settle this statistically would require a great deal of work. Heat causes a slight disturbance compared with pregnancy, and no risk. If the aforementioned view of the nature of the protective influence of heat is correct, it is to be assumed that the changes in the sex glands, proceeding further in pregnancy, are still more efficacious than in heat. The metabolic changes in pregnancy are also greater. If lactation has anything to do with the matter its effect will be added to the other effects of pregnancy. The adrenal glands of the embryo in utero do not appear to be concerned. They are certainly not indispensable, since heat is associated with a marked prolongation of the period of survival. Also, survival much beyond the maximal period in the control animals, has been seen in the pregnant bitch after delivery. In heat and pregnancy it is possible that changes in the uterus may influence the duration of life after total adrenalectomy.

Before leaving the subject of the action of heat and pregnancy, it should be remarked that the discussion is by no means academic. The proestrum or period of the blood-containing discharge in dogs is currently assumed to correspond to menstruation in women. The question will sometimes be asked by patients with Addison's disease, whether menstruation is unfavorable or not in this condition; in any case, it may have to be considered by the physician. Apart from the fact that menstruation may be disagreeable and depressing, especially in the early stages, some patients state that their general condition is distinctly better during menstruation. The question whether pregnancy should be avoided may not arise often, owing to the general condition of the patient. It may sometimes arise, however, or the patient may be pregnant; then there may be the question whether interference should be considered or would be profitable. It would be absurd to claim that our observations on dogs supply data sufficient to settle such questions. But such data as they do supply might be helpful in some measure.

It might be anticipated that in some cases a pregnant animal might react less favorably than a nonpregnant animal to removal of the second adrenal gland. Pregnancy itself being a handicap in certain respects, the surgical operation of adrenalectomy, or possibly the state of adrenal insufficiency may exert an unfavorable influence, so that the period of survival is shortened instead of lengthened. We have seen such instances in dogs. Occasionally, premature delivery may be caused by the operation, although this does not necessarily prevent survival for a longer period than the maximum seen in control animals. In one

dog, labor began half an hour after the operation and the animal survived for twenty-six days, nursing the pups normally. It has not been possible to demonstrate clearly any protective influence of pregnancy toward adrenal insufficiency in cats. Premature delivery is often the result of the operation, which apparently is borne worse by pregnant cats than by pregnant dogs.

It is unlikely that there is an essential difference between dogs and cats in regard to a potential protective influence of pregnancy, although it may be more difficult to demonstrate such an influence in the cat. More attention to the stage of pregnancy at which the second adrenalectomy is made might reveal a period when complications are least likely to occur; a positive effect might then be obtained.

DETOXICATING FUNCTION OF THE ADRENAL GLANDS

An assumed detoxicating function of the adrenal glands has been mentioned already. Those who have supported the idea of such a function have usually conceived of the detoxication as applying to many poisons, both bacterial toxins and drugs. Some have held that the cortex is the tissue concerned in this process; the majority of the authors, however, have considered it a function of the medulla. The evidence brought forward in support of the detoxicating function of the adrenal gland has generally been a supposed increase in the susceptibility of adrenalectomized animals to certain toxins or poisons. As in the case of nearly all other studies made on such animals, investigators have often been misled by their failure to appreciate the influence of unskilled surgical procedures on their results. Tests have been made frequently by introducing highly toxic material or depressing alkaloids into moribund adrenalectomized animals thus hastening death when it is already inevitable.

Claims have been made by a number of authors that animals deprived of their adrenal glands are much more susceptible to the poisonous effects of tetanus toxin and of morphine. A review of the literature may be found in papers from this laboratory by Rogoff and Ecker, and Rogoff and de Necker, who investigated the supposed increase in the susceptibility of adrenalectomized rats to tetanus toxin and to morphine. They found no evidence, in a large series of animals, of increased toxicity of these substances after removal of the adrenal glands. Many rats which had been operated on were able to tolerate practically the same doses of the poisons as were tolerated by control animals which had not been operated on. Fully three fourths of the deaths that result from double adrenalectomy in rats occur within a period of ten days beginning on about the fifth or sixth day after the operation. The administration of any poison during or preceding this period, as reported

by other observers, would obviously lead to the erroneous impression that the substance was more fatal than in control animals. Indeed, a harmless substance introduced during this period could be considered toxic if no consideration were given to the fact that the animal was moribund. When, however, these poisons were administered to rats adrenalectomized during the period of good health (that is, before or after the period mentioned), it was found that doses up to the minimal lethal dose for control animals were tolerated by many adrenalectomized rats. Rogoff and Ecker investigated the complementing activity of the blood serum in rabbits on which adrenalectomy had been performed and found no difference from control animals which had not been operated on.

That toxins, diphtheria toxin, for example, may poison the adrenal glands as they poison other tissues, is, of course, true. Some of them may even show a preference for the interrenal or the chromaffin tissue. A not uncommon reaction of the cortex to infections and intoxications is hypertrophy (or hyperplasia). But none of these facts can be taken as evidence of a special detoxicating function. Now that our investigations have proved that an active substance, extracted from the cortex, can greatly prolong life after total adrenalectomy, this substance (interrenalin) takes its place with the hormones produced by the other endocrine glands, and the function of the cortex is to produce it. The theory of detoxication was largely based on the desire to endow this gland with a function. Now that a function has been indicated and one of great importance, for a substance which preserves life cannot be insignificant, it is probable that the theory will retire into the background.

The last speculation on functions of the cortex which will be mentioned is that it is concerned with lipoid, especially the production of cholesterol, and governs cholesterol metabolism. There is little or no foundation for this theory. Cholesterol, of course, exists in the cortex in a relatively large amount, along with lecithin. The inclusions of birefringent lipoid in the cortical cells, especially in the spongiocytes of the zona fasciculata, are the most prominent microscopic features. They are picked out in polarized light by their property of double refraction. Their impressive appearance does not prove that they play an important rôle. The histologic picture is an uncertain guide as to their function and fate, although some cytologists are convinced that they represent a stage in a process of secretion which culminates in their extrusion into the blood. It cannot be said that any really crucial evidence has been furnished to support this conclusion. We ourselves can only contribute the fact that in our studies on adrenalectomized dogs no definite changes in the cholesterol content of the blood were made out.

THE ADRENAL MEDULLA

None of the endocrine glands produces an active substance better characterized than epinephrine. Its chemical constitution is understood. It has been prepared synthetically. Its physiologic reactions are numerous and striking. It is known to be found in the cells of the medulla. It is responsible for the brown color which the massive inclusions in the medullary cells assume in chromic acid or chromium salts. It has been proved to be given off to the blood, and it is the only substance of the group in which this has been clearly demonstrated. In numerous experiments on dogs, cats, rabbits and monkeys it has never been missed in the blood of the adrenal veins unless its liberation was purposely interfered with. Its passage from the cells to the blood is strictly under the control of nerves running in the major and minor splanchnic nerves and in branches from the lumbar sympathetic chain. When these are completely cut, no epinephrine is found in the blood of the adrenal veins, although the substance can still be found in the medullary cells. No symptoms are produced by section of the nerves supplying the adrenal gland. A temporary loss of weight may occur but this has no special significance. The animals remain in good health indefinitely. The same is true if the medulla is completely extirpated. It is, therefore, impossible to assign to the medulla any function indispensable to life, or, indeed, any function of physiologic importance. This has seemed disappointing to some authors; they have asked whether it is likely that an organ producing and discharging a substance capable of such striking reactions when administered artificially does not possess physiologic significance. One school of authors apparently accepting the view that under ordinary conditions not enough epinephrine is discharged to cause any definite effect, has put forward the hypothesis that in certain emergencies the output of epinephrine is markedly increased through stimulation of the secretory nerves of the medulla. Certain reactions associated with emotions are supposed to be brought about largely through epinephrine liberated by the adrenal glands. These reactions can be elicited by stimulation of sympathetic nerves and also by epinephrine in sufficient doses. The only question is whether sufficient quantities of epinephrine are liberated per unit of time, under the stress of these emotions, to raise the concentration of that substance in the arterial blood to the level at which stimulation of the structures involved will occur. If such quantities are liberated, it is scarcely necessary to verify the occurrence of the reactions.

We believe that there is no evidence that it makes any difference whether the adrenal glands are discharging epinephrine, are prevented from discharging it, or are absent: the reactions accompanying emotions are the same, quantitatively and qualitatively. We are therefore unable to accept the view that an important, or indeed a perceptible factor, in emotional expression is an increased output of epinephrine.

CHANGES IN OUTPUT OF EPINEPHRINE

The same considerations apply to the supposed increase in the rate of output of epinephrine caused by stimulation of certain afferent nerves and by general asphyxia. If the rate of liberation of epinephrine is sufficiently increased, any of its physiologic reactions can of course be obtained. In experiments, however, which permitted a quantitative estimation of the epinephrine given off per minute by the intestine segment method, we were unable to find any increase either during stimulation of the central end of the sciatic, the brachial or the splanchnic nerves, or during asphyxia. Stimulation of the peripheral end of the splanchnic nerve caused a large increase, as much as twentyfold, whereas excitations of the central end, interspersed among the stimulations of the peripheral end, had no effect on the rate of output. Numerous reflexes were elicited by the stimulation of the central splanchnic nerve, while it failed to affect the output of epinephrine. We have repeated these experiments on afferent stimulation in several researches, always with the same negative result; and similar results were obtained for asphyxia. Acute cerebral enemia, however, caused by tying off the arteries going to the head, excites an increased output of epinephrine, as was first demonstrated by Rogoff in this laboratory.

It would be inappropriate to use the present occasion for controversial purposes. The only reason for mentioning that our results on certain points differ from those of some other investigators is that the discrepancies involve points of technic, including operative technic, which have been found to influence greatly the consequences of adrenalectomy. Differences in the results of experiments on the survival of animals which always die after removal of the adrenal glands depend essentially on differences of technic, including of course preoperative and postoperative care. The decision must always be in favor of the investigator whose animals live longest, assuming, of course, that adrenalectomy was complete and accessories were absent. There is no reason why discrepancies in the results of acute experiments should not also be due to differences in the operative work. If one observer, for instance, fails to obtain a reaction (for example, acceleration of the denervated heart or shrinking of the denervated limb on stimulation of the sensory nerves) when the adrenal glands are eliminated, while another succeeds in obtaining it, the decision must be in favor of the latter who has been able, by better technic, to eliminate the glands without abolishing the reaction. The best method is to perform the operation, partly or wholly, some time before the experiment, which is made after complete recovery from the operation. In any case, we consider that extreme care must be exercised in removing the adrenal glands. Clipping off the veins tightly enough to prevent passage of blood from the glands is the least harmful procedure for those whose

experience in these operations is not extensive. This does not abolish the reaction of the limb caused by stimulation of the sciatic nerve, although it can be verified that the block on the adrenal veins is complete. The same considerations apply to operations on the adrenal glands in the experiment on the denervated heart. The crucial test is to perform the experiment after proper elimination of the glands. We have obtained the reactions, often in undiminished intensity, in their absence. It is, therefore, impossible for us to believe that it is correct to attribute the reactions solely to the reflex discharge of epinephrine. If the reactions can be typically elicited with the glands lying in a dish, the adrenals are not essential to their production.

The positive observations on the influence of sensory stimulation and of asphyxia reported by certain Japanese observers who used our intestinal segment method, have been thoroughly considered and checked by us. We may report on this matter in another paper. We have no hesitation in stating that their conclusions are based on an imperfect application of the method. Their values for the concentration of epinephrine in the blood of the adrenal veins and, therefore, in the outputs, are loaded with a serious error which makes them much too great, incredibly great to us, in many instances. We believe that this is due to the cumulative effect of more than one mistake in the application of the method. In some cases, acceptance of the nominal concentration of epinephrine in the standard instead of the real concentration may be a factor. Material loss of epinephrine from solutions made up at the beginning of the test and kept standing, with frequent opening and introduction of pipets into the bottles, can occur. We make up each specimen of epinephrine just before application to the segment. If an error of this nature is made, the effect will be to increase the apparent concentrations (and outputs), and the observer who obtains the smaller concentrations may be presumed to have avoided error from this cause more successfully than he who obtains the larger concentrations. When one of the Japanese observers finds a concentration of 1 : 250,000 in the initial sample of blood from a dog, weighing less than 12 Kg., with a flow of blood of 30 cc. per minute from the adrenal glands, we can only say that there is a big error somewhere. We have never seen any condition like this in the large number of dogs on which we have made estimations. The output for the animal per minute is 0.12 mg. of epinephrine (the observer gives it as 0.012 mg., but this is obviously an error if the concentration is given correctly). If this output were maintained for eight or ten minutes only, an amount of epinephrine equal to the entire store in the glands would be given off. He does not point out this amazing result or make any comment on it; it simply goes down in the table. When another of the Japanese investigators finds in a cat a concentration of 1 : 40,000 in the blood of the adrenal vein, probably about

1 : 20,000 in the serum (after nicotine), no suspicion is betrayed that results of this kind tend to discredit the paper. Yet in discussing reports of other authors on perfusion experiments with the adrenals of oxen (a poor method for studying the secretion of epinephrine) a point of exclamation is inserted by the observer when he mentions that concentrations of from 1 : 100,000 to 1 : 20,000 (!) were found in the perfusion fluid. The point (of exclamation) is well taken. In a deafferented dog, that is, one in which the posterior roots supplying the operative field have been cut, a concentration of 1 : 70,000 was found in the initial specimen of blood, with a correspondingly large output. Nothing like this was found in the rest of the experiment, even when sensory nerves were stimulated. The explanation given is simply that the dog was excited and objected to being fastened. No such concentrations and outputs were found in initial specimens from other dogs of the series which behaved in the same manner. There are numerous records of excessive concentrations in practically every table in the series of papers, concentrations such as we have not seen under similar conditions, and many of which could be estimated by a colorimetric method. In our own investigations, it was only when the output was greatly stimulated (by nicotine) that concentrations capable of being estimated colorimetrically were seen, although they were much smaller than those given by the Japanese investigators. On internal evidence, therefore, we must decline to accept the results of these workers; we object as strongly to the figures by which they profess to confirm our work (strychnine, nicotine, etc.) as to the figures on which they rely when they come to conclusions different from ours (sensory stimulation and asphyxia).

Other observers who have obtained what they consider positive results have used methods which did not permit estimation of the rate of output of epinephrine or of changes in that rate (anastomosis of the adrenal vein of one dog with the jugular vein of another, etc.). Their conclusions are based on the occurrence of one reaction or another which they interpret as indicating an increase in the output of epinephrine during sensory excitation or asphyxia. But without exception the supposed demonstrations fail to show that the output is increased. A great defect in these methods is that changes in the rate of blood flow from the adrenal gland and the rate of passage of epinephrine into the reacting animal are not controlled.

ACTION OF DRUGS ON THE OUTPUT OF EPINEPHRINE

The action of certain drugs, first investigated by us, on the output of epinephrine has been mentioned incidentally. It deserves fuller treatment for its physiologic interest and, in some instances, because of a possible therapeutic value. Space is not available, however, for

a detailed discussion. It will suffice to say that some drugs increase the rate of output, for example, strychnine. The increase may be to many times the initial value, and the arterial blood may become charged with detectable concentrations. The action of strychnine is central; section of the splanchnic and other nerves going to the adrenal glands prevents it. An increase to 100 times the original (diminished) output in an animal with a high section of the cord has been seen on administering strychnine. Morphine (in cats) and physostigmine are other drugs which increase the output.

Other drugs diminish the output of epinephrine. Nicotine is an example. The paralyzing action is preceded by a brief period (from half a minute to a minute) during which the output is greatly increased. The increase swings over quickly into a marked decrease, which lasts much longer but can end in a recovery if the dose is not too great. The stimulating action can be elicited after the splanchnic nerves, etc., have been cut; it is, therefore, not a central action.

A large number of drugs may be expected to exert an action on the output of epinephrine. We investigated a considerable number and had planned to examine a good many additional drugs likely to be found active, but the work on the cortex came to the point at which all our available time was absorbed. It was necessary to abandon further work on the action of drugs on the output of epinephrine at least for a time. Whether such an increase as can be caused by strychnine and other drugs would ever be useful could only be determined by experience. Calculation, however, of the greatest possible effect of safe doses on the output might indicate whether the corresponding increase in the concentration of epinephrine in the arterial blood would be of any use in certain conditions. It is easy, of course, to introduce artificially far greater amounts of epinephrine than can ever be given off from the glands. Usually the results have been disappointing. That epinephrine should have no substitutive effect in experimental adrenal insufficiency, as shown by us many years ago (for the first time, we believe, on properly prepared animals), and in Addison's disease, is to be expected since the loss of the cortex is the important lesion in these conditions.

COMMENT AND SUMMARY

Our first step was to show conclusively that the interrenal tissue (cortex) is the part of the adrenal body indispensable to health and life. The chromaffin tissue (medulla) can be destroyed, the discharge of epinephrine from it arrested by section of its nerve supply, or both operations can be performed without ill effect, immediate or remote, and, indeed, without any noticeable effect.

We then studied the symptomatology and pathology of the condition which follows complete adrenalectomy (total adrenal insufficiency)

and determined the length of the period of survival and the period of good health in a large series of dogs, destined to serve as controls for our studies on the influence of treatment by cortical extracts and in other ways. Nothing in the literature was found to be of value, because it was clear that the animals had died largely in consequence of inadequate operative technic and seldom, if ever, from adrenal insufficiency alone. Our results proved that dogs properly operated on, and under proper conditions, lived in a majority of the cases from 6 or 7 to 8 or 9 days. A good many survived well into the second week, and not a few lived for 13 or 14 days. Occasionally a dog lived a day or two longer. The average for the first 120 dogs was 7 days, and for the later series about 8 or 9 days.

Cats live longer than dogs, the average period of survival in our series being 11 days. Many cats live for 2 weeks; a few 3 weeks. Occasionally an animal survives longer than the fourth week. One lived for 32½ days.

A series of animals in which treatment is given (for example, with extracts), in order to yield a definitely positive result, must show a fair number of animals surviving well beyond the maximal periods of life in the control series; also, a considerable number should reach the longer periods of survival of the control animals. A comparison of the averages is not at all satisfactory unless the tests mentioned give strongly positive reactions. If a series of treated cats were to show a few animals living from three to five weeks and many of them living from nine to fifteen days, this could not be regarded as a positive result. Similar results for dogs, however, would be considered positive. Since the comparison depends absolutely on the control animals, too much care cannot possibly be taken in accumulating a good control series.

We have shown for the first time that extracts of cortex, from the adrenal glands both of dogs and of animals from the slaughter-house, cause prolongation of life when injected into dogs, far beyond any results seen in the control animals. The period of good health is correspondingly prolonged. The interrenal tissue therefore contains and, it is to be supposed, produces a substance which we suggest may appropriately be termed interrenalin; and this substance has the power of staving off death in adrenalectomized dogs. As soon as the difficulty of this feat is realized, the question whether life can be prolonged ten days, fifty days or hundreds of days ceases to take first place. The problem becomes a matter of dosage, the preparation of the extract, etc. If life is prolonged beyond a doubt by the action of an extract, the active substance in some way or other must enable the organism to surmount all the emergencies which threaten its life and will inevitably destroy it when it has reached the span, brief at best, allotted to dogs deprived of the adrenal glands and not subjected to

treatment. It is not a small reaction of an unimportant substance which neutralizes the poisons destined to overwhelm the untreated animal within a week or two, or prevents their development by contributing to the metabolism what is necessary to keep it normal; nor is it a small reaction of an unimportant substance which staves off the breakdown, probably centering about the gastro-intestinal tract, and announced first of all among the serious terminal symptoms by the onset of anorexia. It is a potent reaction of a highly significant substance that takes hold of a dog destined to die, let us say at the end of the tenth day, and carries him on in good health until the end of the twentieth or thirtieth day, or sometimes much longer. The test is, indeed, so severe that it ought not to have occasioned disappointment had the series in which treatment was given rarely revealed an animal living longer than any of the controls. There would still have been good reason to believe in the existence of a cortical hormone and the possibility of substitution of it for the missing interrenal tissue. Clear and definite proof, however, which must be accepted by everyone would not have been obtained. It is quite another thing to study a symptom not in itself acutely fatal in order to see whether treatment with extracts has any effect on it. This could be done with insulin by estimating the sugar content of the blood, but nothing of the kind is possible, at present, in the case of the active substance of the cortex. It is true that we have gained the impression that some of the symptoms, especially the anorexia and what often characterizes it, the aversion to fats, may be modified by treatment. But this is too vague to serve as a crucial test. Nor does it seem possible at present to employ the development of the increase in the nonprotein nitrogen, the urea nitrogen, etc., as a test of the efficacy or inefficacy of a treatment, for the change occurs when the severe terminal symptoms appear or are about to appear; when these symptoms show themselves there is no reason for troubling about less evident changes.

It would be premature to attempt to decide at present what the function of "interrenalin" is. Pharmacologic studies may throw some light on the question. Its influence on the general metabolism or on some special metabolic process must, of course, be investigated. The fact that in the untreated animal, as has been mentioned, the breakdown seems to center around the gastro-intestinal tract and that the pathologic changes are prominent there suggests that the cortex has some special relation to that tract, including the pancreas. In Addison's disease, gastro-intestinal symptoms are among the classic features. We have seen at autopsy in a case of acute adrenal insufficiency a condition (gastro-intestinal congestion, hemorrhage, blood in the lumen and congestion of the pancreas) precisely resembling what is so often observed in dogs dying after loss of the adrenal glands. The necropsy showed destruction and atrophy of the glands. The relationship, however, is not asserted; it is

merely suggested as a hypothesis. It is uncertain whether the asthenia, which develops sooner or later following the appearance of anorexia, is so specific that the cortex must be assumed to bear a special relation to the neuromuscular system.

Whatever the function of the cortex may be, the artificially administered extracts substitute for it, so that the animal, so far as is known, remains entirely normal. It is possible, however, that even from the first day, cumulative changes occur, although they escape detection, which in the long run, culminate in the abrupt appearance of the terminal symptoms and death.

If it is the office of the cortex to contribute to, perhaps to control, the functioning of certain tissues or organs or certain metabolic processes, these will, of course, suffer derangement after removal of the adrenal glands. In addition to the loss of these functions and, of course, connected with it, the development of metabolic faults or faults of intestinal absorption may lead to the accumulation of poisons. In fact, a marked feature in animals dying of adrenal insufficiency is an intoxication, usually revealing itself some time after the anorexia has appeared, deepening as the end approaches, and finally submerging the central nervous system and affecting the circulation. One can think of loss of the cortical active substance, therefore, as influencing the organism in two ways: derangement of the mechanisms or processes which it normally controls, and the development of poisons owing to that derangement. These two factors are not mutually exclusive. An analogy may be found in diabetes. Lack of insulin not only interferes with normal carbohydrate metabolism, leading to an increase in the sugar content of the blood, but also leads to the accumulation of substances associated with the deranged metabolism, which may cause diabetic coma. That an intoxication is present in total adrenal insufficiency is, in any case, certain. The condition can be relieved, as we have shown, by the intravenous injection of salt solutions. These obviously cannot supply a hormone, and may act by washing out injurious substances. Dogs have been kept alive by this treatment for as long as fifty-four days after removal of the second adrenal gland. Many lived far beyond the maximal period seen in the controls. Some were rescued after serious symptoms (for example, coma) had developed, and survived for many days thereafter.

It is shown that no function indispensable to life is performed by the medulla. Indeed after the destruction or denervation of the medulla, or both, everything goes on as before; the animal lives on indefinitely in good health and is indistinguishable in its behavior from an animal which possesses its adrenal chromaffin tissue. One deduction from this fact is that there is no foundation for the view, almost invariably expressed in clinical writings, that Addison's disease is due to loss of

epinephrine. Some physiologists, on the strength of ambiguous observations interpreted as indicating a reflexly increased output of epinephrine, have unduly stressed, we think, the importance of the part played by the medulla in vascular reactions and in the reactions associated with the expression of emotions. If no change is caused by elimination of the medulla, epinephrine from the medulla does not exercise important functions.

A detectable content of epinephrine has always been found by us in the blood of the adrenal vein, although too small for detection in the arterial blood owing to the great dilution. The output of epinephrine is much increased by stimulation of the peripheral end of the splanchnic nerves, according to our quantitative confirmation of previous workers. It is greatly diminished or abolished by section of the nerves going to the adrenal glands. Whether the epinephrine secretory nerves regenerate after section cannot be stated definitely; after many months, the output of epinephrine is still much diminished. The concentration of epinephrine in the blood of the adrenal veins is approximately in inverse ratio to the blood flow, so long as the flow is not too small. The output is markedly increased by certain drugs, for instance, strychnine, physostigmine and, for a brief time (usually a minute or less), nicotine. It is diminished by other drugs, such as nicotine (following the brief increase). It is increased, although not beyond the maximum of the ordinary output (in cats), by acute cerebral anemia.

The foregoing observations have been confirmed by subsequent investigators. While we have been unable to measure a clear difference in the output of epinephrine during the excitation of sensory nerves or during general asphyxia, some other observers claim to have done so.

It may be asked, what then is the function of the medulla? In the past, errors have been made in attempts to provide this gland with a function. I should be loath to conclude that it is the "roi fainéant" of the endocrine glands, a kind of personage little known to physiology. To say that it must be doing something, is a proposition which surely is not worth while contradicting. It may even appear self-evident to some. The real question would be, is it doing anything worth while, anything that can properly be spoken of as a function? Does it keep up the blood pressure? The blood pressure, however, is not altered, either during or after removal of the glands. The point of view is sometimes shifted by the assumption that the extracapsular chromaffin tissue is doing the same thing as the medulla, turning out the necessary epinephrine, and that epinephrine is not lacking after elimination of the adrenal glands. Those who believe that reactions, such as those previously mentioned, are abolished when the glands are eliminated, can hardly acquiesce in this conception. There is experimental evidence against it. When more is known of the formation of epinephrine and

its forerunners, new light may be thrown on the question under discussion. We cannot accept lightly the view that the medulla has no physiologic function, but is important only as raw material for the manufacturing druggists.

There is evidence that in certain forms of experimental hyperglycemia, epinephrine from the adrenal glands may be a factor, although usually a minor one. Thus, puncture at a certain part of the floor of the fourth ventricle causes hyperglycemia and also an increased output of epinephrine. The hyperglycemia is well obtained when the adrenal medulla has been eliminated, and is dependent on splanchnic nerve fibers going to the liver. How much it is aided, if at all, by the increased output of epinephrine, dependent on splanchnic nerve fibers leading to the adrenal glands, depends merely on the rate and duration of the increased output. If the concentration in the arterial blood is increased beyond the threshold value needed to cause epinephrine hyperglycemia and is maintained above this value long enough, a part of the effect will be due to the epinephrine. The hyperglycemia associated with asphyxia does not seem to have an adrenal element. No difference has been found in the amount of the hyperglycemia whether the medulla was eliminated or not. On the other hand, the hyperglycemia caused by morphine (in cats and dogs) is greatly diminished or abolished when the adrenal medulla is eliminated. Such observations suggest, although they do not prove, that epinephrine may exert some influence on carbohydrate metabolism. But it must be remembered that, so far as we know, the concentration of epinephrine in the arterial blood never under physiologic conditions approaches the level at which it would itself cause an increase in blood sugar. There is no evidence that epinephrine from the adrenal glands is the factor which causes hyperglycemia when the function of insulin is interfered with. Hyperglycemia in the depancreatized dog is well marked after elimination of the adrenal medulla.

In considering the question of functions of the medulla it should be kept in mind that no important function can in any case be assigned to an organ the loss of which causes no symptoms. It is not so with the thyroid and the parathyroid glands, the islets and the adrenal cortex; their physiologic value is revealed by the consequences of their removal. They may, therefore, be assumed and are known to exercise important functions. It may seem curious that the adrenal medulla is abundantly supplied with nerves which control the output of epinephrine strictly, while it has not been proved that the production and discharge of the important hormones of the thyroid and parathyroid glands and islets are under the control of nerves. The adrenal cortex functions normally when the gland is denervated. If, as is currently believed, the adrenal medulla is composed of cells representing genetically sympathetic ganglion cells, it is to be expected that it should differ from

the other endocrine glands enumerated, for all of them are of quite different origin. The pituitary gland is left out of the discussion; little is known about its functions. Genetically, the anterior lobe would fit in with some of the other endocrine glands but not with the adrenal medulla.

We have suggested as a possibility that the reason for the close control of the output of epinephrine by the nervous system may not be to keep up the concentration in the arterial blood to a certain beneficial level, but to keep it below a certain harmful level. In the adrenal glands, there exists a stock of epinephrine which under abnormal conditions (for example, massage) can be liberated, not all at once, but so rapidly that harmful concentrations might be reached. This stock, amounting usually to about one-thousandth of the moist weight of the adrenal glands, possibly 1 per cent of the moist weight of the medulla, could easily be dangerous were it possible for it suddenly to escape into the adrenal veins.

It is impossible to go into the interesting observations, first put on a sound basis by Elliott, on the store of epinephrine. This, of course, represents a balance between production and discharge; its amount gives no direct information as to changes in the rate of output. Intense and prolonged emotional disturbance causes no effect on the store of epinephrine, but numerous drugs diminish it greatly.

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On the Function of the Adrenals.

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Three-quarters of a century ago, Thomas Addison (1) established the relationship between a syndrome, which now bears his name, and pathological changes in the adrenal glands. He made clinical and post mortem observations upon eleven cases, which have since been

abundantly confirmed. The importance of the adrenals for life and health became an object of many experimental investigations on the physiology and pathology of these organs but, thus far, our knowledge of their function remains quite obscure. The vast literature that has accumulated upon the subject is out of proportion to the amount of substantial information it has yielded. Unfortunately, most of the work has been so poor that its mention can only be regarded as evidence of the conscientious efforts of many authors to notice everything which has been published. In this article, no attempt will be made to engage in extensive citation or review of the literature, especially on the earlier investigations, as this is available to the reader in the exhaustive book, by Professor Biedl, on the Internal Secretions (2).

I shall limit myself chiefly to a brief review of some of the investigations in which I have been engaged since 1915, in collaboration with Professor G. N. Stewart, and reference will be made only to some papers that have a direct bearing upon our work. The object is to emphasize clearly the difference in relative importance between the functions of the interrenal tissue, or cortex, and the chromaffin tissue, or medulla, of the adrenal glands. It is not sufficiently recognized, at present, that functionally, as well as anatomically, these two divisions of the gland, though topographically related, are to be considered as two different glands. The adrenals being indispensable organs for life, it is not surprising that epinephrin assumed an important rôle when it was found to be a product of these glands. Its striking pharmacological reactions having been demonstrated at a time when little was known about the cortex, it was natural that this substance should have been vested with the indispensable function of the adrenals. While this view is still maintained by many authors, we are convinced that epinephrin secretion from the adrenals can be dispensed with, causing no harmful results, and that the important rôle is played by the interrenal tissue. Our investigations support the view that the indispensable function of the interrenal tissue depends upon the elaboration of a hormone (Interrenalin).

Experiments on the function of the adrenal cortex or interrenal tissue.

Shortly after the publication of Addison's observations, a number of investigators attempted to create adrenal insufficiency in animals, by extirpation or destruction of the glands. These attempts always led to very early death of the animals, often after excision or destruction of only one adrenal. In the experiments of Brown-Séquard (3) none of the animals died from loss of the adrenals alone. All the animals, including 44 rabbits, 9 guinea pigs, 3 rats and a number of cats and dogs, died in 9 to 37 hours after double adrenalectomy. It is obvious that inadequate technique was chiefly responsible for the short period

of survival from the fact that in a group of animals, consisting of 16 rabbits, 5 guinea pigs, 2 cats and 2 dogs, all died within 24 hours after removal of only one adrenal. Brown-Séquard arrived at the conclusion that the adrenals are indispensable for life, but it must be stated that while this conclusion happens to be correct, it can only be considered as a fortunate guess. We cannot accept his results, or the results of most of the more recent workers on adrenalectomy, as having established the indispensability of the adrenals.

Many years later, when knowledge of the principles and practice of surgery and the use of anesthetics was sufficiently advanced to permit the application of adequate surgical technique in performing adrenalectomy, the results obtained by others were not much better. Strehl and Weiss (4) were able to improve upon the results obtained with dogs and some cats, when they allowed an interval of a month to elapse between removing the first and second adrenal, but in general their results are poor. The survival periods obtained by them, when both adrenals were removed at one operation, were 22 to 75 hours in 7 dogs, 75 to 138 hours in 3 dogs, 15 to 28 hours in 15 cats, 28 to 47 hours in 2 cats, 8 to 14 hours in 26 rabbits, 4 to 9 hours in 20 guinea pigs and 15 to 19 hours in 4 rats. When the adrenals were removed, with an interval between excision of the first and second gland, 4 dogs survived 109 to 124 hours, 5 cats lived 30 to 170 hours and 9 rabbits died in 21 to 76 hours. These must be considered very unsatisfactory results, when compared with those which will be discussed in connection with our own observations. More recently, there have been experiments reported that are fully as bad as the earliest attempts at adrenalectomy. Thus, in the experiments of Blodinger, Klebanoff and Laurens (5), most of the dogs died in 8 to 24 hours. Bornstein and co-workers (6) made chemical and clinical studies upon animals surviving only a few hours.

It cannot be expected that studies made upon moribund animals will yield valuable information concerning adrenal insufficiency. When an investigator has reported observations on rabbits and rats, as well as on other animals, it is possible to estimate the skill of the operator from his results. In the observations reported by Lewis (7), 3 dogs died within 12 to 15 hours after adrenalectomy, 8 rabbits in 15 to 60 hours and 20 to 40 per cent of the rats died in the first 48 hours, obviously from surgical causes. It is surprising that anyone should attempt to determine the toxicity of certain substances upon such animals. The fact is well established that adrenalectomised rabbits and rats survive much longer, and that a large proportion of these animals can survive indefinitely. I shall not discuss the many other examples of unreliable experimental data afforded by most of the work on this subject. It is too obvious from such work that death of the animals, within a relatively short time following adrenalectomy, must be regarded

the result of unskilled surgery, since it occurred before the conse-
iences of loss of the adrenals could have supervened.

Our results have been so far superior to any of those reported by her investigators that we can explain them only by the extensive ex- rience and practice which has enabled us to develop the necessary ill and technique for performing adrenalectomy properly. The inti- ate relationship of the adrenal with important nervous structures as well the vena cava, in all laboratory animals except the rat, compels the erator to acquire a particular skill and technique, in order to remove e gland without introducing serious complications which contribute early death of the animal. Except in rats, there can be no object in moving both adrenals at one operation. There is little doubt that e short survival periods obtained by other operators is largely due excessive traumatization of the important nerve structures that have timate anatomical relationship with the adrenals, to prolonged an- thesia, and to other, less obvious, surgical factors. These handicaps e minimized by permitting an interval to elapse between the removal the first and second adrenal, and by developing sufficient skill to able the operator to perform the operation in a very short time. e have usually permitted an interval of one to two weeks to elapse tween operations and have found no difference in our results after iger intervals. We have operated upon large numbers of dogs, cats, bbits, rats and guinea pigs, to determine the survival periods follow- g removal of their adrenals. The data, thus obtained, constitute ntrol series essential as a starting point for other studies on adrenal sufficiency. From time to time new controls are added to the series, the purpose of determining whether greater experience or modifi- tions in technique influence the survival periods among our controls. rats the operation is so simple, and anatomical conditions favorable, at there is no advantage in performing the operation in two stages. the other animals, however, it is desirable to permit a sufficient erval to elapse between excision of the first and second adrenal, to able injured nervous structures on one side to recover before inflict- g similar injury on the opposite side. Adrenalectomy is much easier cats than in dogs, rabbits or guinea pigs, but even in cats there is advantage in performing double adrenalectomy in one operation.

Rabbits and rats are not useful for most studies on adrenal in- fficiency since a large proportion of these animals survive, indefinitely, e loss of both adrenals. Among different groups of rabbits we have served from 25 to 40 per cent of indefinite survivals and among rats average of about 50 per cent. It has been assumed that the presence accessory cortical tissue, in many of the animals, is responsible for eir longer survival. Although this is quite probable, it does not enti- y explain the survival of a large number of animals in whom careful arch fails to reveal visible accessory bodies. At any rate, we have

preferred to make most of our studies upon dogs and cats, since in these animals accessory cortical bodies are rarely met with and complete removal of both adrenals invariably leads to death of the animals as the result of adrenal insufficiency, in the absence of accessory cortical tissue. Whenever we have discovered an accessory body, at the time of excision of an adrenal, it was removed in the same operation.

Guinea pigs have not proven as useful as dogs or cats for most of our work. Their resistance is not so great and mortality is sometimes high as the result of intercurrent or epidemic diseases. The incidence of accessory cortical tissue is less common than in rats or rabbits and more common than in dogs or cats. In a series of 60 male guinea pigs, we found accessory cortical tissue in 9 animals. In one, it was discovered and excised during the operation for removal of the first adrenal. Two of the animals survived $41^1/_2$ and 43 days, respectively, and the accessory bodies were found at necropsy. The remaining six animals were sacrificed 6 to 8 months after they had been adrenalectomised and post mortem examination revealed the presence of accessory cortical bodies. It is interesting to note that a long interval (about 3 months) was allowed to elapse between the first and second adrenalectomy, in this series of guinea pigs. It is probable that during this interval hypertrophy of the accessory cortical deposits occurred in consequence of loss of one adrenal, and that this continued after the second gland was removed. Of the remaining animals in this group, 2 died of intercurrent disease and 50 succumbed to the loss of their adrenals. In these, careful search failed to reveal the presence of accessory cortical bodies. They survived as follows: — two lived somewhat less than 3 days, one into the 4th day, nine into the 5th day, fourteen into the 6th day, ten into the 7th day, three into the 8th day, eight into the 9th day, one into the 12th day, one into the 17th day and one a little over 17 days.

We have recently published (8) a control series of adrenalectomised cats. In about 50 animals the average period of survival was 11 days. Few of the cats survived less than a week, more than one-fifth of the total number lived 2 weeks or longer, one survived nearly 30 days, one nearly 32 days and a castrated male cat lived over 35 days. As in the case of our results on dogs, these periods of survival are much greater than any hitherto reported by other investigators. The animal which survived over 35 days had been castrated long before it was brought to the laboratory and as another castrated male that came into our possession survived double adrenalectomy only a week, we are unable to state whether this circumstance in any way modified the survival period.

The dog offers the most favorable conditions for studies on adrenal insufficiency. Its superior intelligence, compared with other laboratory animals, enables the observer to make satisfactory clinical

idies, the larger size renders it less difficult to obtain urine by cathe-rization and sufficient blood may be safely withdrawn for chemical aminations. We have accumulated about 150 control animals in our ries of adrenalectomised dogs. Our intention was to acquire 100 ani-als for this purpose. About 50 additional animals have accrued, thout preparing them to be used as controls, from experiments upon e influence of various forms of treatment which proved ineffective. ry few of the animals died in less than 4 or 5 days. The large majo-y lived more than a week and a number of them survived up to 14 16 days. In paper I (9) of our series of articles on adrenal insuffi-ncy, we have published, in 2 tables, the results on 34 male and female control dogs and in paper V (10) a table of 36 additional ntrols. The average period of survival is more than a week. There is difference between males and non-pregnant females or females not rut.

We have observed (11) that pregnancy affords protection against e consequences of adrenalectomy, in dogs. Of 17 pregnant adrenal-omised dogs, one survived for nearly 59 days, a period equal to the erage gestation period. Another lived into the 58 th day, one into the th day, one into the 33 rd day, one into the 27 th day, one into the th day and one into the 23 rd day. Six survived from 13 to 18 days. e survived into the 26 th day, after giving birth to a litter of pups mediately after the operation for removal of the second adrenal. e observation on the last animal mentioned indicates that presence the foetal adrenals does not offer an explanation for the longer rvival of pregnant animals after adrenalectomy. In this connection is of interest to mention our experience with dogs adrenalectomised ile in the condition of rut (12). All the dogs that were deprived of second adrenal, while in this condition, survived much longer than maximum period of the controls. One lived into the 22 nd day er adrenalectomy, one survived 32 days, one into the 37 th day and into the 65 th day. The suggestion arises that the longer survival iod observed in pregnant dogs might be explained by the protec-influence afforded by the condition of rut, which preceded preg-ncy. We have not made any observations on the influence of rut in renalectomised cats. Thus far, we have not been able to demonstrate favorable influence of pregnancy in this animal. Adrenalectomy, in gnant cats, is usually followed by abortion, which often results in rtening the survival period of the animal. Not infrequently, this has n observed after the operation for removal of the first adrenal in s and sometimes in dogs. Pregnancy, though capable of exercising rotective influence in the absence of the adrenals, must also be consi-ed a serious handicap in the operation for adrenalectomy. The bable influence of the corpus luteum suggests itself from our ob-vations on adrenalectomy during pregnancy and rut in dogs. The

influence of these conditions, upon the survival period of adrenalectomised dogs, points to a functional interrelationship between the adrenal cortex and reproductive organs, which has already been suggested by histological observations and by our knowledge of the ancestry of the interrenal tissue.

Untreated adrenalectomised dogs continue in good health up to about 2 to 3 days preceding death, regardless of the period of survival. During the period of good health the animal cannot be distinguished from any healthy unoperated dog. It is vigorous, eats well and continues to exhibit any habits or special tricks that it was capable of performing before being deprived of the adrenals. Body temperature, blood pressure, pulse and respiratory rates are unaltered. Blood examination reveals no significant change in the red and white cell counts, percentage of hemoglobin, electrical conductivity of serum, relative volume of corpuscles and serum, non-protein nitrogenous constituents, cholesterol, calcium, chlorides or sugar content, until the development of the symptoms which presage the fatal outcome. Sometimes changes are found in the blood a day or two before the onset of symptoms, but this is not the case in the large majority of animals.

The period of decline begins with development of anorexia and this is usually preceded by an aversion to food rich in fat. The animal may select lean pieces of meat or other contents of the dish, avoiding fat. On the following day there is usually total anorexia, although if something in the nature of a delicacy (chicken, salmon or other food not ordinarily included in the diet) is offered, the animal can be coaxed to eat a small amount, once or twice, refusing even that kind of food thereafter. Vomiting frequently occurs, becoming bilious in character and blood is commonly found in the stools, as the animal rapidly declines. During this period the animal generally becomes apathetic and somnolent but rarely has more than transient asthenia. However, events progress somewhat rapidly at this stage and within the ensuing day the animal becomes much more somnolent and decidedly asthenic. As the symptoms develop, the blood pressure falls and heart block has been frequently observed, the heart becomes irregular, usually quite slow. Respiration is slow and shallow and may become of the Cheyne-Stokes type shortly before death. Coma is present, sometimes for a number of hours preceding death. Frequently, there are phenomena referable to the nervous system. Hallucinations develop, the animal stares as if in alarm and this is associated with fits of yelling and racing about in the cage. These fits generally last for a few minutes and may be repeated a number of times daily until the animal's condition has progressed to the stage of somnolence and asthenia. Not seldom, there is seen muscular twitching, increased excitability of reflexes and tetanic convulsions.

The principal changes that we have observed in the blood (13, 14) during development of the terminal symptoms consist of marked increase in the total non-protein nitrogen, which is largely, but not entirely, due to increase in the urea nitrogen. There is also a decided increase in the so-called undetermined fraction of the non-protein nitrogen, while the creatine, creatinine, uric acid and amino acid nitrogen remain practically unaltered. The calcium content of the blood is usually elevated, cholesterol is unaltered and the blood sugar frequently shows a moderate fall, especially shortly before death, but not enough to suggest any relationship between hypoglycemia and the convulsions which occur. The chloride content of the blood is often moderately diminished. Concentration of the blood occurs, often preceding the onset of symptoms, and continues increasing till death. There is an increase in solids, specific gravity, hemoglobin percentage, erythrocyte count and in the relative volume of cells. The serum is diminished in relative volume but its specific gravity and protein are unaffected.

Necropsy generally reveals a striking condition of the alimentary canal. The stomach usually contains a considerable amount of bile, frequently also blood. Often, the entire small intestine is filled with bloody material. The mucous membrane of the entire alimentary canal is considerably congested and in some places profoundly hemorrhagic, ulcers being common in the stomach and sometimes present in the duodenum. Other organs show no significant gross changes except the pancreas, which almost invariably is markedly congested. The clinical condition described, the blood changes and post mortem findings point to the development of a very severe and rapidly fatal intoxication. It is surprising that no manifestations of any change in the animal can be recognised during the relatively long period of survival preceding the onset of symptoms, yet once they occur their severity rapidly increases and death follows in a relatively short time.

Having acquired the information on the survival period, symptomatology and period of good health of adrenalectomised animals, afforded by our large number of controls, we were enabled to make further studies on adrenal insufficiency. The consequences of loss of the adrenals indicate retention of toxic material in the body. Further evidence of this was obtained from our observation that life can be much prolonged in these animals by intravenous administration of relatively large quantities of physiological salt solution (15). Obviously, the loss of a hormone is not compensated by this treatment. We have suggested that the beneficial influence is the result of dilution of toxic material and facilitation of its elimination as the result of introducing the liquid into the circulation. In a series of 17 animals thus treated the period of survival, in most of the animals, was far beyond the maximum seen in our control series. The majority survived from 20 to 54 days after complete adrenalectomy. Often, we have observed marked amelioration

of symptoms, during and following the injections, and sometimes, animals that had already developed coma were rescued and kept alive, in good health, for a long time.

Among the earliest theories of adrenal function, the suggestion was included that the glands are concerned in the destruction, neutralization or elimination of toxic material. Aside from the evidence of intoxication resulting from the absence of the glands, however, experimental work has failed to yield substantial proof of a "detoxicating" function of the adrenals. Some investigators have endeavored to prove an increased toxicity of certain drugs or bacterial toxines, in adrenalectomised animals, but the experiments were far from convincing. The principal defect in nearly all of them is the failure to obtain adrenalectomised animals in sufficiently good condition to render them suitable for such tests. It has not been realized, by some writers, that administration of a highly toxic substance or a depressing drug to an animal whose life hangs in the balance may easily lead to error in interpretation of the influence of toxic substances.

We have tested the alleged increase in susceptibility of adrenalectomised white rats to tetanus toxine (16) and to morphine (17), and have found no support for the claims made by certain other investigators (7). In a large series of control rats, we have shown that fully three-fourths of the animals that succumb to loss of their adrenals, and in which no accessory cortical bodies were found post mortem, die within a period of 10 days beginning about the 5th day after the operation. Curiously enough, certain observers have been unable to realize that the mortality rate of adrenalectomised rats will be much higher, if during this period a depressing drug like morphine is administered. Nevertheless, we have seen recovery after doses of morphine up to the lower limit of the M. L. D. If morphine is administered within a few days after adrenalectomy has been performed some of the animals recover from the effects of the drug and die some days later (usually within the above mentioned period) from adrenal insufficiency. Of course, if an operator cannot get many animals to survive more than 1 or 2 up to a few days after adrenalectomy, any substance when introduced, in any dose, into such animals will appear to be more toxic since all must die shortly.

The most probable explanation of the function of the adrenal cortex rests in the view that, like other endocrine glands which have been more successfully studied, it elaborates a hormone which is indispensable for life and health. We have obtained evidence which indicates the existence of such a hormone and are directing some of our efforts toward its extraction from the adrenal cortex (10, 18). We have obtained extracts, prepared in various ways from the cortical portion of adrenal glands, which were found to be effective in markedly prolonging the life of adrenalectomised dogs. The extracts were administered intra-

:nously, in doses of 0.5 cc. to 1 cc., in some cases daily and in others ı alternating days. The earlier experiments were performed with pre-ırations made from dogs' adrenals, obtained aseptically during ope-tions for adrenalectomy. Later, we extended our observations on aterial obtained from the slaughter house. About 25 per cent of the ıimals, thus treated, survived well beyond the maximum period of ırvival of our control dogs. One animal lived nearly 79 days. Many the animals survived for periods approaching the maximum for con-ols. Not infrequently, we have observed amelioration of symptoms ıd modification of the pathology found post mortem. We do not con-der an extract to be effective, however, unless it is capable of pro-nging life in a substantial proportion of the animals, well beyond the aximum period of survival of our controls.

Conclusions should never be drawn from comparison of averages the survival periods of two series, since the averages of quantities hich vary as much as the survival periods are not of much value, pecially in small groups of experiments. Nor is it permissible for an server to compare the average period of survival of his "treated" ıimals with the average for controls selected from the literature, as ıs recently been done. Since, as previously mentioned, pregnancy and ıt have a favorable influence upon the survival period of adrenalecto-ised dogs, we employ only males for experiments upon the efficacy treatment. Our test is a severe one, for the only criterion available, present, to determine the value of adrenal extracts is prolongation of e and the period of good health, of completely adrenalectomised ani-als, beyond the maximum of untreated controls. Nothing, our experiments, can explain this prolongation of life except the esence, in the adrenal extracts, of something which for a time can ıbstitute for the specific substance produced by the adrenal cortex.

We have administered some of the extracts (in capsules, usually ated to resist gastric digestion) as treatment, in a number of cases Addison's disease and have observed beneficial influence. Of urse, a longer time and more cases will be required to determine what rmanent benefit can be obtained. For, although the syndrome is the sult of impaired adrenal function, it is commonly associated with or ıe to the presence of other unfavorable conditions, most often tuber-ılosis, which cannot be expected to be directly influenced by admini-ration of adrenal cortical extracts. In estimating the benefits observed these cases, it is essential to recognize the possibility of temporary ontaneous improvement of the patient, or psychological effects simu-ting benefit due to the treatment. But it is significant that when im-ovement is seen, it occurs about 2 or 3 weeks up to 4 or 5 weeks ter the beginning of treatment. The beneficial influence of adrenal rtical extracts in the treatment of Addison's disease must, of urse, vary with the amount of functioning adrenal cortex still present

in the patient and indirectly with the severity of underlying or associated conditions. Addison's disease is not identical with the condition seen in adrenalectomised dogs, where there exists total acute suppression of adrenal function without associated serious complications. Nevertheless, certain symptoms, though varying in severity and character, are commonly observed both in Addison's disease and in completely adrenalectomised animals. Some of these symptoms are anorexia, aversion to fatty foods, gastro-intestinal disturbances (including bilious vomiting), muscular asthenia and not seldom symptoms referable to the nervous system, especially in acute conditions. We have seen amelioration of these symptoms under treatment with our extracts.

The low blood pressure in Addison's disease, which has hitherto been interpreted as an indication of interference with the epinephrin secretion from the adrenals, we believe is probably a result of deficient cortical function, perhaps resulting from an influence of the gradually developing intoxication upon the circulatory organs. In view of the available knowledge, from the better experimental and clinical studies, we must consider Addison's disease as a manifestation of disease of the interrenal tissue rather than the chromaffin tissue of the adrenal glands. If lack of an indispensable hormone is responsible for the syndrome it is the interrenal hormone and not epinephrin. If the hormone is lacking, it is extremely likely that an intoxication would develop which might be relieved by administration of products obtained from the organs which elaborate the hormone. An excellent example of this is afforded by the use of insulin in diabetes.

It will be shown under "experiments on the function of the adrenal medulla" that the epinephrin secretion from the adrenals does not exercise an indispensable function. The fatal consequences of bilateral extirpation of the adrenal are due to loss of the cortical function. Our experiments upon the prolongation of life in completely adrenalectomised animals and the observations upon Addison's disease, under treatment with adrenal cortical extracts, yield substantial confirmation of the view that the indispensable function of the adrenals is the elaboration of a hormone by the cortex of the glands. To distinguish this hormone from adrenalin and to indicate its origin from the interrenal tissue, we have suggested the name "Interrenalin".

Experiments on the function of the adrenal medulla.

The observations of Oliver and Schafer (19), demonstrating that extracts of the adrenal glands are capable of causing a marked rise in blood pressure, when injected into the circulation of animals, was followed within a few years by the isolation of an active principle, adrenalin, having the same physiological influence. The discovery of this substance, at a time when very little information on the interrenal

ssue was available gave support to the theory that the adrenals secrete hormone and adrenalin became recognized as the indispensable product of the glands. Numerous investigations followed, on the elaboration, storage and secretion of this substance.

In view of the striking pharmacological reactions obtained by administration of adrenalin (in doses much larger, however, than the mounts liberated by the adrenals) theories of adrenal function were ntered about assumed functions of its secretion from the glands. The fluence of adrenalin upon the circulation together with the observaon that in Addison's disease there exists a condition of low blood essure led to the supposition that the adrenals are concerned in the aintenance of normal pressure in the circulation, through the action the secretion from the medulla of the glands. However, though it ay seem disappointing, the evidence afforded by the best experimental ork fails to support this view. Whatever may be the function of pinephrin, it does not exercise an indispensable rôle in the body, since s secretion can be suppressed or abolished without affecting the life health of the animal. Blood pressure remains unaltered in the bsence of epinephrin secretion from the adrenals. This has been deonstrated by studies made upon adrenalectomised rabbits (20) and ogs (21), using a modification of Van Leersum's (carotid loop) ethod for making blood pressure measurements upon unanesthetized nimals. No significant change in the pressure occurred, during relatily long periods of survival in good health, until the development of e symptoms which terminate in death of the animal.

Frequently, investigators have been misled by speculation based pon the pharmacological reactions of adrenalin, when physiological terpretations were made. Since the quantities of adrenalin employed, eliciting pharmacological reactions, are much greater than the amounts hich correspond with the probable physiological secretion, the reacons observed from such quantities (often toxic doses) can only lead error if physiological interpretations are made from them. Obviously, e most reliable information concerning the function of a secretion is btainable from quantitative observations on the ordinary rate of its beration, the conditions that are capable of modifying that rate and e possible physiological effects of the secretion under the conditions udied. For this purpose, it is, of course, essential that the method nployed in the investigation should be capable of yielding satisfactory uantitative information.

Inadequate methods have often been employed by some workers the investigation of this subject. It seems pertinent, therefore, to call the mathematical conditions which govern the measurement of a elocity. To measure the rate of a secretion the method must be pable of permitting the measurement of a mass and a time. If this not possible, the method is not correct in principle and information

yielded by it cannot be reliable. Many of the studies on epinephrin secretion, reported in the literature, have been conducted with methods that could not yield quantitative information, yet quantitative interpretations have been confidently made from reactions that are not even specific qualitative reactions for epinephrin. Certain denervated organs (heart, pupil, limb) have been employed as test objects for epinephrin. While these structures are capable of reacting to adrenalin, the reactions which have been relied upon as indicating epinephrin discharge from the adrenals are obtainable in the animals, as well or sometimes even stronger, after they have been deprived of the glands by proper methods. These test objects, however, can be employed satisfactorily if adrenal vein blood is collected for a known time, then released into the circulation at a known rate when no other factor except the circulating epinephrin might be responsible for the reaction obtained. The quantity of epinephrin liberated can then be determined by injection of known amounts of adrenalin introduced at the same rate. But this has not been the manner in which the reactions have been employed by those who have depended upon these denervated organs as reagents for demonstrating increased epinephrin secretion from the adrenals. Thus, Cannon (22) has relied upon acceleration of the denervated heart during asphyxia or stimulation of the central end of the sciatic nerve, as an indication of a large increase in the amount of epinephrin discharged from the adrenals, because he was unable to obtain these reactions when the adrenals were excised. The same interpretation was made by Anrep (23) from reflex volume changes in the denervated limb. Although we have often obtained these same reactions fully as well after depriving the animal of its adrenals, it may be pointed out that even if it were true that these reactions are abolished by suppression of the epinephrin secretion, it could not be assumed that an increased liberation of epinephrin was responsible for the reaction. It would merely indicate that epinephrin was essential in obtaining it. We suggested that blood, containing the ordinary amount of adrenalin, flowing through the sensitized organs at a higher rate (as a result of increased blood pressure) would offer to the tissues a larger amount of epinephrin in a given time, without a change in the rate at which it is liberated into the blood from the adrenals. Anrep and Daly (24) have objected to this explanation on the basis of the view that pharmacological reactions depend upon the concentration of a drug in the blood and not upon the amount circulating. That may be the case where drugs are not sensibly used up in making the circuit of the reacting tissues or when much more than the minimum concentrations required to produce the reactions are employed. On the other hand, when a substance, like adrenalin, is used up in passing through the tissues, where it exerts its effect, the average concentration in the circuit will be greater if the flow is increased. Epinephrin reactions have been

tained in the denervated pupil with subminimal quantities of circulat-g epinephrin, by causing more blood to pass through the eye when ternative paths in the circulation were clamped off (25).

We have examined the alleged influence of asphyxia and sensory imulation upon the liberation of epinephrin from the adrenals and ive been unable to find proof of an increase produced in this manner. ie claim has never been supported by satisfactory measurements of e rate of epinephrin secretion. The conclusion was originally drawn om experiments that could not have yielded reliable information. They nsisted of withdrawal of blood, from a cat, through a catheter which is inserted through the femoral vein into the vena cava, up to a level ar the adrenals. The cava blood was then applied to a strip of cat's testine and a very crude qualitative test for epinephrin was made (26). ven if the epinephrin concentration of the blood had been determined antitatively no information could have been obtained on the rate of liberation from the adrenals, for the method does not permit the easurement of the rate of blood flow from the adrenals or through e vena cava. Later, the denervated heart was adopted by Cannon an indicator for epinephrin, but measurements of the rate of secretion ere not made before, during or after asphyxia and sensory stimulation.

Failure to obtain acceleration of the denervated heart when the renals are excised must be interpreted as an indication of improper moval of the glands. We have repeatedly observed this reaction (and e reactions of other denervated organs that have been relied upon indicators for epinephrin secretion) when we suppressed the epi-phrin secretion or removed the adrenals. Sometimes a reaction may l to occur immediately after the adrenals have been excised. However, the animal has not been subjected to excessive traumatization or shock ring adrenalectomy the reaction returns in a short time. It is, there-re, not trustworthy as an indicator for epinephrin. Explaining the action obtained by us in the absence of the adrenals, as has been ggested by Professor Cannon, that the liver, thyroid and other gans may contribute to, or cause, acceleration of the denervated heart, rtainly does not strengthen confidence in the reaction as a quanti-tive method for measuring changes in the rate of epinephrin output. rthermore, no change in the output can be detected under asphyxia sensory stimulation if the adrenal blood is prevented from entering e circulation and is collected at a known rate of flow in a dish at the me when the acceleration of the heart occurs and the inephrin concentration in the blood is determined (27, 28). If the eged large increase in the output exists we ought to be able to detect in the adrenal blood captured at the moment the outburst of epi-phrin is supposed to occur. By the "cava pocket" method we are able determine, with certainty, relatively small as well as large changes either direction in the epinephrin output. Consequently, failure do

detect a change, under the conditions mentioned, must be considered as satisfactory proof that it does not exist.

In any case, the supposed reflex augmentation of epinephrin secretion can have no significance unless it is shown to be capable of exerting physiological reactions. It has been maintained in support of the theory of an "emergency function" of the adrenals that, in times of stress, the major emotions (anger, fear, rage), painful stimulation, etc., are associated with outbursts of epinephrin from the adrenals. The epinephrin, thus liberated, is supposed to effect a mobilization of defensive mechanismus in the body enabling combat, flight, etc. The heart becomes accelerated, circulation increased, pupils dilated, hairs become erect, blood coagulation is hastened and sugar is mobilized in the blood to facilitate greater muscular activity (all of which have been observed under the influence of pharmacological doses of adrenalin). No evidence has ever been obtained, by any of the proponents of the emergency theory, that the adrenals can liberate epinephrin in sufficient quantities to cause such reactions. We have shown that they occur in the absence of the adrenals or in animals that have been subjected to an operation for suppression of the epinephrin secretion. Furthermore, adrenalectomised animals, during their survival in good health, and animals surviving indefinitely after epinephrin secretion has been suppressed, by excision of one adrenal and a major portion of the other (including the medulla) with denervation of the remaining cortical fragment, are as capable of combat or exhibition of their emotions as normal animals. They could not be distinguished from unoperated, healthy animals.

The only convincing manner in which to demonstrate any change in the epinephrin output from the adrenals is to employ a method which permits measurement of the rate of secretion before, during and after the introduction of any condition whose influence upon the rate is to be tested. In our studies this has been accomplished. We collect adrenal vein blood through a cava pocket for a given time, measure the quantity of blood obtained and determine the rate of blood flow from the glands. The epinephrin concentration in the blood specimens is then determined, using a segment of rabbit's intestine, and often confirmed on a segment of rabbit's non-pregnant uterus, as first employed by G. N. Stewart (29). This information renders possible the calculation of the rate of epinephrin secretion. By this method we have made many satisfactory measurements of the rate of spontaneous liberation of epinephrin from the adrenals and have determined a number of conditions that are capable of modifying that rate as well as conditions that are without detectable influence upon it.

In large series of cats and dogs and in a small number of monkeys we have determined the average rate of liberation of epinephrin from the adrenals at 0.000225 mgm. per minute per kgm. of body weight (30). In cats, the average output was somewhat lower (0.0002 mgm.) when

ether was employed as an anestetic than (0.00025 mgm.) when urethane was used. The rate does not differ when the adrenal vein blood is obtained via the extraperitoneal lumbar route and when the abdomen is opened in the usual manner for preparing the cava pocket in the same animal (31). An increase of the epinephrin output, up to 10 or 12 times or more, can be obtained by stimulation of the splanchnic nerve (32). The output can be diminished or abolished by denervating the glands (33, 34). With a favorable segment of rabbit's intestine, we were able to determine, in some experiments, that the liberation of epinephrin from the adrenals, after denervation, could not have amounted to $^1/_{1000}$ of the initial rate. No reaction was obtained with the segment although it was determined that it could have detected such an amount if it existed. Suppression of the epinephrin secretion occurs when transection of the spinal cord is performed in the upper thoracic region and hemisection of the cord, at this level, affects the secretion from the adrenal on the same side (35, 36). Certain drugs are capable of modifying the rate of epinephrin secretion; thus, strychnine (31) or physostigmine (38) cause a marked augmentation of the epinephrin output, while curare (39) diminishes it. Morphine (40), in cats causes a large increase but in dogs it produces little or no effect upon the epinephrin output. Nicotine (41) causes a large, brief increase (lasting one minute or less) followed by marked depression of the epinephrin output. If the dose is not large there is a gradual return to, or near, the initial rate of secretion as the blood pressure approaches its previous level. Thus, it is obvious that the cava pocket method as employed in our studies can yield reliable quantitative data on the rate of liberation of epinephrin from the adrenals. Certain Japanese investigators have repeated some of our studies using our method. In some instances they drew the same conclusions as we did, in others they differed. Space will not permit detailed discussion of the matter but it must be stated that there is evidence in their papers that they have not always employed the method properly. Failure to recognize the limitations of the method has sometimes led them to faulty interpretations. Our extensive experience with the method leads us to rely upon it as the best available method, when properly employed, for quantitative studies on the epinephrin secretion from the adrenals.

It has been alleged that an important relationship exists between epinephrin secretion and carbohydrate metabolism. This has not been supported by satisfactory experimental evidence. There is no foundation for the statement that glycogen storage in the liver is interfered with in the absence of the adrenals (42). Nor can it be assumed, from pharmacological reactions of adrenalin, that hyperglycemia is associated with or dependent upon epinephrin secretion from the adrenals. Certain experimental hyperglycemias (piqûre, ether, asphyxia) are readily obtained in animals whose epinephrin secretion has been suppressed or

whose adrenals have been excised (42, 43). Indeed, all of the reactions that we have thus far investigated and which have been alleged to be due to the influence of epinephrin are obtainable after the secretion has been abolished.

No significant change occurs in animals as the result of loss of the epinephrin secretion. It must, therefore, be concluded that, whatever the function of epinephrin may be, this substance does not play a very important rôle in the body and that the indispensable function of the adrenals rests in the cortex. Unfortunately, the cortex of the adrenal has received less attention than the medulla because the latter afforded more striking pharmacological reactions for study. It must be realized, however, that, while the epinephrin secretion offers many interesting problems for solution, the questions concerning the indispensability of the adrenals can only be solved by acquiring knowledge of the functions of the cortex of the gland.

Summary.

Under ordinary experimental conditions the average rate of epinephrin secretion from the adrenals is 0.00022 mgm. per minute per kgm. of body weight. This rate can be augmented or diminished by stimulation of the splanchnic nerve or denervation of the glands, respectively. The rate can be modified by administration of certain drugs. Changes in the rate of epinephrin secretion, under these conditions, have been determined quantitatively by the "cava pocket" method with the aid of rabbit's intestine and uterus segments for measuring the concentrations of epinephrin in adrenal vein blood. This method when properly employed fulfills the mathematical conditions which govern the measurement of a velocity, i. e. the measurement of rate of secretion. It is, therefore, correct in principle and yields unambiguous results. Circumvention of these conditions is incompatible with the elaboration of reliable information. These conditions are not fulfilled in the various methods that have been often relied upon to prove changes in the epinephrin output, especially when the test object employed is not even a specific qualitative reagent for epinephrin. Large outbursts of epinephrin secretion that have been alleged to occur under the influence of reflex excitation or during emotional states, have never been proven to exist. All the reactions that have been relied upon to prove such outbursts can be obtained in animals that have been deprived of their adrenals or after suppression of the epinephrin secretion from the glands. Certain theories of adrenal function, based upon the epinephrin secretion, are without satisfactory experimental foundation. There is no evidence that the epinephrin secretion from the adrenals exerts an important function in the body. It can be completely suppressed in animals without causing harmful effects.

The fatal outcome of complete adrenalectomy is the result of loss f the adrenal cortex and not the medulla. A severe intoxication deve- ɔps toward the end of the period of survival in animals that have been eprived of their adrenals. This intoxication can be relieved and life rolonged considerably by intravenous administration of relatively large mounts of physiological salt solutions. Marked prolongation of life an be obtained in completely adrenalectomised animals by administra- on of certain extracts prepared from the cortical portion of adrenal lands. There is evidence that a functional interrelationship exists etween the adrenal cortex and organs of reproduction. The indispen- ıble function of the adrenals, however, may be explained by our ex- eriments which indicate the elaboration of a hormone (Interrenalin) y the interrenal tissue.

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DIAGNOSIS AND TREATMENT OF ADDISON'S DISEASE*

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Introduction

AS an introduction to my remarks on Addison's disease, I shall quote the first and last paragraphs in the preface of the article by Thomas Addison[1] entitled, "On the constitutional and local effects of disease of the suprarenal capsules." He states, "If pathology be to disease what physiology is to health, it appears reasonable to conclude that, in any given structure or organ, the laws of the former will be as fixed and significant as those of the latter, and that the peculiar characters of any structure or organ may be as certainly recognized in the phenomena of disease as in the phenomena of health." Recognizing the fundamental importance of basing an understanding of pathology upon a thorough knowledge of physiology, he concludes, "There are still, however, certain organs of the body the actual functions and influence of which have hitherto entirely eluded the researches, and bid defiance to the united efforts of both physiologist and pathologist. Of these, not the least remarkable are the 'suprarenal capsules', the atrabiliary capsules of Casper Bartholinus; and it is as a first and

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1930

feeble step towards inquiry into the functions and influence of these organs suggested by pathology, that I now put forth the following pages.''

The accurate observations and interpretations made by this keen clinical investigator on the pathology of the suprarenal capsules laid the foundation for studies on the physiology of these glands. Despite the labours of a large number of investigators, which have resulted in an enormous literature, progress has been very slow in unfolding the rôle of the adrenal bodies, upon which must be based a satisfactory concept of diseases of the organs and their treatment.

In the consideration of the diagnosis and treatment of Addison's disease I shall present a new conception of the subject, based upon physiological and clinical studies on adrenal insufficiency, that I have been conducting during the past fifteen years at The H. K. Cushing Laboratory of Experimental Medicine. The view that has heretofore prevailed, and is still adhered to by a number of excellent clinicians who have occasion to study and treat this condition, is based upon the assumption that Addison's disease is chiefly a manifestation of absence or failure of function of the epinephrin (adrenalin) secretion. Our experimental studies have yielded substantial evidence that this syndrome develops in consequence of absence or lack of function of the *interrenal* (adrenal cortex) tissue rather than interference with the function of *epinephrin* secretion (from the adrenal medulla).

Historical

In 1855, when Addison called attention to the existence of the syndrome, he described a group of symptoms that are associated with disease of the adrenal glands. The significance of these

symptoms, from the standpoint of interpreting the physiological disturbances which follow the loss of adrenal function, has remained quite obscure. Experimental investigation and clinical observation has, until recently, added very little knowledge of practical value in understanding and treating this disease. Much confusion has resulted from failure to consider the adrenal body as being composed of two glands, different in origin, structure and function, though anatomically united. In some lower animals (certain fishes) they exist as separate structures, the *interrenal bodies* corresponding to the cortex and the *chromaffin tissue* to the medulla of the mammalian adrenals.

The indispensability of the adrenal glands for life and health was evident from Addison's observations. His report led to experimental studies upon animals by a number of investigators. Surgical removal of the glands, or complete destruction by mechanical means and by cautery led to early death of the animals. These results were interpreted as confirmation of Addison's observations on the indispensability of the glands. They also led to the obvious suggestion that the glands either supply an indispensable internal secretion or that they exercise a detoxicating function, destroying certain substances which, in the absence of the glands, accumulate in the body and cause death.

In 1895 it was found by Oliver and Schafer[2] that adrenal extracts, prepared by rubbing the glands with salt solution, if introduced into the circulation of an animal, were capable of causing a striking elevation in blood pressure. These investigators observed that this effect is caused only by extracts made from the medulla of the glands, the cortical portion being ineffective. Shortly thereafter the active pro-

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1930

duct of the medulla, adrenalin, was isolated and prepared in pure form. Since then it has been commonly accepted that adrenalin (epinephrin*) is the indispensable hormone of the adrenal gland, the lack of which is responsible for the condition described by Addison. This followed quite naturally from the knowledge that adrenalin has a powerful influence in raising blood pressure and that in Addison's disease the blood pressure is low. I will point out later, however, that this idea is not supported by satisfactory experimental and clinical evidence and that treatment of Addison's disease based upon this conception does not yield beneficial results.

Personal Observations

The secretion of epinephrin from the adrenals and the conditions under which it is liberated into the blood has been extensively studied by us. Under ordinary experimental conditions it is liberated from the glands at a constant rate (average, 0.000225 mgrm. per kg. of body weight per minute).[3] This rate can be influenced through the splanchnic nerves, stimulation causing an augmented epinephrin secretion, and section of the splanchnic, interference with the secretion.[4, 5] Similar influences upon the epinephrin output from the adrenal glands can be produced through the action of certain drugs.[6, 7, 8] It has not been demonstrated, however, that this secretion exercises an indispensable function, although we have observed that it is capable of evoking certain physiological reactions, when liberated at the ordinary rate.[9] The best evidence at present available, indicating that epinephrin is

* It is preferable to employ the term "epinephrin" as indicating the physiological secretion from the adrenal medulla, and to use "adrenalin" when referring to the commercial product.

a physiological secretion, can be interpreted to support the view that it may be a toxic product, elaborated by the adrenals for elimination at such a rate as is necessary to permit its destruction without exerting injurious effects. Of course, the possibility exists that, although elaborated as a waste product it may perform a function before its elimination. In this sense CO_2 may be suggested as an example.

However, when considered in view of our knowledge of the products of other endocrine organs, it is most probable that epinephrin is a physiological secretion and that it is liberated at a constant rate to perform a definite function which, however, is not indispensable for life and health. Various theories have been proposed, to explain the function of epinephrin, but none of them are supported by sufficient and convincing experimental evidence. Prominent among these is the so-called "emergency theory," proposed by Cannon. According to this theory, pain, fear, anger or the exhibition of the major emotions in any emergency results in a large outpouring of epinephrin from the adrenals, which is capable of mobilizing the defensive mechanisms of the individual. The experimental evidence upon which the theory was founded is very meagre. The method employed by Cannon and his co-workers[10, 11] to study the epinephrin secretion (withdrawal of blood from the vena cava through a catheter inserted through the femoral vein) could not be expected to yield quantitative information on the rate of epinephrin discharge from the adrenals. Since this was pointed out by Stewart and Rogoff, Cannon and his pupils have vigorously defended the theory, relying chiefly, however, upon other indirect evidence, instead of adopting methods which would enable them to offer

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OF MEDICINE
1930

quantitative data upon the subject which is necessarily a quantitative problem. In any case, the principal physiological reactions relied upon by Cannon to prove increased epinephrin secretion from the adrenals can be obtained in animals in which the epinephrin secretion from the glands has been suppressed.

The claim that large increases in the rate of epinephrin secretion can be elicited reflexly by stimulation of the central end of the sciatic or brachial nerves, and by asphyxia, has not been supported by satisfactory experimental evidence. We have been able to demonstrate that the spontaneous liberation of epinephrin from the adrenals is governed by a centre or centres in the upper thoracic cord.[12] The existence of a spinal nervous mechanism would suggest the possibility of reflex influences upon the discharge of epinephrin from the adrenals. However, the usual experimental methods for exciting reflexes fail to demonstrate an increase in the rate of epinephrin secretion, when properly tested by actual measurements. The method employed by us for such measurements is capable of detecting significant changes in the rate of secretion if they exist. As already stated we have measured changes in the rate caused by the action of drugs and by splanchnic stimulation as well as by a number of other means. Our only conclusion can be that significant reflex increases in epinephrin secretion have not been possible to demonstrate, claims to the contrary, based upon indirect evidence or unsatisfactory experiments, notwithstanding.[13, 14]

The important, if not the only, purpose of a theory should be to stimulate investigation. To this end the various theories concerning adrenal function have been very useful. It is amazing, however, how often theory is confused with fact and the readiness with which

equivocal experimental evidence is often accepted without question. This would not be so serious if limited to matters of academic interest, but it is to be deprecated when applied to the practices of medicine and surgery. Thus, it should be considered a very dangerous procedure to perform adrenalectomy in human beings for relief of certain circulatory and other disturbances, when this is done on assumptions based upon theory or upon very unsatisfactory and equivocal experimentation.

Finally, it must be emphasized that although much interesting and valuable work has been done on the secretion of epinephrin from the adrenals, its function, if it has one, is not indispensable. For it is possible to completely suppress the secretion of epinephrin in animals without causing any detectable harmful influence.[5, 15] This can be accomplished in certain animals (rabbits, rats) by double adrenalectomy. A relatively large proportion of such animals survive indefinitely the loss of both adrenals. In these there is usually found one or more small accessory bodies, consisting entirely of cortical substance. Complete removal of both adrenals, of course, results in total suppression of epinephrin secretion from the glands. Another manner of abolishing the epinephrin secretion is the removal of one adrenal and a major portion of the remaining gland. In addition, the remaining fragment is completely denervated. So long as a small portion of the cortex remains in connection with its circulation it will suffice to sustain life indefinitely and maintain the animal in good health. Removal of this cortical fragment results in death within the same period as when total extirpation is performed. There is evidence, also, from post-mortem examination of some cases of Addison's disease, where only

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1930

a part of the cortex and none of the medulla of the adrenal must have been present for some time, that death ensued when the remaining cortex was destroyed or its circulation blocked. In such patients, excepting possibly during a severe acute exacerbation, and in animals that have been subjected to suppression of the epinephrin secretion and are surviving in good health, there is no evidence of any lack of capacity for exhibition of the emotions, under appropriate conditions.

It is established, by convincing evidence, that the cortex (interrenal tissue) is the indispensable portion of the adrenal gland. We have also demonstrated that this tissue contains a substance which can be extracted and is capable of prolonging life in adrenalectomized animals.[16] Further, I will present satisfactory evidence that Addison's disease is the consequence of lack of function of the adrenal cortex, not the medulla, and that the extract which I have prepared from the cortex is beneficial in treatment of patients with Addison's disease, while adrenalin (from the medulla) is either harmful or of little if any real benefit in cases where the diagnosis is quite certain.

Thus far it has not been possible to produce in laboratory animals a condition identical with Addison's disease in human beings. Total extirpation of the adrenal glands results in acute suppression of function, and partial removal of the organs results either in death, the same as with complete removal, or in recovery. If a sufficient amount of cortex to sustain life is present symptoms do not develop. Removal of anything less than the amount which suffices to sustain life is equivalent to complete ablation. I have already stated that death is due to loss of the cortex and not of the medulla. It is not

surprising that adrenalectomy in animals does not produce a condition identical with Addison's disease in which suppression of cortical function is practically always a chronic process and, in a large proportion of cases, complicated by serious underlying or associated pathological conditions.

Although loss of cortical adrenal function is invariably fatal, adrenalectomised animals when properly operated upon survive much longer than has generally been supposed. All of the older reports in the literature, and, indeed, nearly all of the recent ones are unreliable. They merely demonstrate that the operation for adrenalectomy should not be undertaken by anyone unless he possesses excellent surgical skill and develops adequate technique by extensive experience. The anatomical relations of the adrenal with important structures, particularly sympathetic nerves and ganglia, render the operation for the proper removal of the gland extremely difficult. Traumatization, excessive anæsthesia, etc., may introduce serious complications. It is obvious that in reports found in the literature, adrenalectomised animals surviving but a few hours to one or two days did not die from loss of the adrenals alone but largely as the result of poor surgery. The large number of studies made upon such animals can have no value, and whenever an interpretation from such studies is later proved by satisfactory work to have been correct the interpretation can only be considered as a fortunate guess.

The animal of choice for studies on adrenal insufficiency is the dog. In this animal accessory adrenals are rarely encountered and its size permits withdrawal of satisfactory samples of blood, when desired for chemical studies. The intelligence of the dog also renders it easier to make studies on the clinical condition of the

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Proceedings of the California Academy
of Medicine
1930

animal. In our experience dogs survive the loss of both adrenals for an average of about 12 days, the maximum period of survival being approximately two weeks.[17] No such long periods of survival are to be found in the literature on adrenalectomy performed by other investigators. We have established this control period of survival on over 200 dogs, and our studies upon these control adrenalectomised animals constitute a basis for our studies on the influence of various methods of combating adrenal insufficiency. Following the operation there is a relatively long period of good health during which the animal behaves like any healthy dog that has not been operated on. Within about one to three days preceding death a train of symptoms develop which in many respects are similar to those seen during acute exacerbations in human cases of Addison's disease. The earliest of these symptoms is anorexia, beginning generally with an aversion to fats, and leading to total loss of appetite in a day or two. Up to this time adrenalectomised animals show no change in blood pressure, but as the terminal symptoms develop the systolic and diastolic pressure become lower, diminishing each day till the end.[18] Accompanying the total anorexia the animals usually show signs of severe gastro-intestinal disturbances. There is, frequently, vomiting, becoming bilious in character; diarrhœa is not uncommon and bloody stools are often seen. Muscular asthenia gradually develops and increases during the remaining two or three days, until death. Often there are evidences of severe disturbances in the nervous system. These symptoms usually begin with evidences of hallucinations, the animal developing fits of yelling, racing about in the cage aimlessly, and staring as if alarmed. Muscular twitching is not uncommon and often increases

in severity leading to repeated tetanic convulsions, the animal becoming comatose. The heart is sometimes very slow, at other times rapid, and frequently there is disturbance in rhythm. On post-mortem examination profound congestion and often severe hæmorrhages are found in the mucosa of the entire alimentary canal. Ulcers in the stomach and upper duodenum are quite common, and an almost constant finding is marked congestion of the pancreas. During the period of good health of the animal no detectable changes are found by chemical examination of the blood and urine. With the onset of the symptoms there is usually found concentration of blood, the total NPN is increased, gradually increasing to a considerable percentage toward the end; the urea N also is increased but not in proportion to the total NPN. The undetermined fraction of NPN is relatively high. There is no significant change in the creatine, creatinine, uric acid and amino-acid N. The blood calcium is usually increased and chlorides diminished; blood sugar is frequently diminished, especially toward the end of the period of survival, but the hypoglycæmia is rarely low enough to account for convulsions.[19, 20]

Our studies upon control adrenalectomised animals indicate the development of a severe and rapidly fatal intoxication. We have found[21] that life of adrenalectomised dogs can be prolonged well beyond the maximum period of survival of untreated animals by intravenous administration of relatively large quantities of physiological or isotonic salt solutions (up to 54 days). Obviously, nothing in such solutions could substitute for a specific hormone that is normally elaborated by the adrenal gland. Increasing the survival period by this treatment can only be due to dilution of toxic substances

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OF MEDICINE
1930

and facilitation of their elimination or "washing out". Probably also, to some extent there may be a beneficial influence upon the circulation.

Having obtained sufficient experimental evidence to leave no doubt that the condition which develops, and leads to a fatal outcome, following the loss of both adrenals is due entirely to loss of the interrenal or cortical structure, we continued our studies in an attempt to obtain from the adrenal cortex a product which could substitute for the loss of the gland. After a number of attempts, carried on over a period of years, we were finally able to demonstrate satisfactorily that certain extracts prepared from the cortex of the adrenal, when introduced intravenously in small quantities, once daily or on alternating days, are capable of prolonging life in adrenalectomized dogs well beyond the maximum period of survival of control animals.[16] The quantities of the extracts introduced was usually from 0.5 to 2 c.c. Such amounts are much too small to have contributed any beneficial influence from the introduction of fluid into the circulation. Obviously, the beneficial influence of these extracts could be due to nothing else than supplying that substance which was lacking in the animal as the result of the loss of its adrenals. Since the substance is a specific product of the interrenal tissue we have adopted as the name for this hormone "Interrenalin", which designates its origin.

In my studies on human beings with Addison's disease I have been able to correlate clinical observations with years of experience in animal experimentation on adrenal insufficiency. It has previously been indicated that the condition which follows double adrenalectomy in animals is not identical with the condition seen in human beings who have

Addison's disease. In the latter case the onset is more gradual, the condition is more chronic and is very often associated with other underlying conditions or complications, most frequently tuberculosis. As already stated, in the adrenalectomized animals the condition is much more acute and the suppression of adrenal cortical function is total. Nevertheless, though differing in degree, certain symptoms already described or mentioned in the description of the condition which develops in adrenalectomized animals are very commonly seen in patients with Addison's disease, for example, muscular and circulatory weakness, gastro-intestinal disturbances, and disturbances referable to the nervous system, including hallucinations, convulsions and coma. I have observed in nearly all my patients with Addison's disease that they have an aversion to fatty foods. This is a common symptom occurring in adrenalectomized dogs and is of interest in view of the previously mentioned condition of the pancreas found at post-mortem examination of animals that died in consequence of removal of the adrenal glands. In Addison's disease of very short duration, where there was an acute suppression of adrenal function or acute destruction and degeneration of the glands, I have seen at autopsy similar, though less intense, congestion of the pancreas and of the gastro-intestinal mucosa.

Diagnosis

The diagnosis of Addison's disease, when all of the symptoms described by Addison are present, should present very little difficulty. Yet, it is surprising how often a classical picture of this malady escapes recognition, doubtless because the condition is comparatively rare, and the clinician who has not a special

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1930

interest in this disease is not on the look out for it. It must be stated, however, that if properly recognized it would be found that the condition is more common than has been generally supposed. It has often been stated, in the past, that Addison's disease is rarely diagnosed before autopsy, I can hardly agree with this statement although it might be considered not without foundation from the number of relatively easy diagnoses that have been missed and from the comparatively large number of cases that have been referred to me as Addison's disease which proved to have been improperly diagnosed. I am convinced that a number of claims of "cure" in cases of Addison's disease fall into this class. I do not, however, deprecate the diagnosis of Addison's disease in doubtful cases when symptoms warrant suspicion and cannot be accounted for by differential diagnosis. In such a case it is preferable to err in favour of this diagnosis, giving the patient the benefit of early treatment, should the diagnosis prove to have been correct. Nevertheless, I am satisfied that not only can the condition be usually diagnosed with certainty but that the pathology of the adrenal glands can often be predicated from the clinical symptoms and the course of the disease. I have demonstrated this to a number of colleagues in Cleveland by suggesting the probable pathology some time before death in a number of cases in which my prediction was confirmed at autopsy. I might add, also, that in a number of cases that were presented to me as Addison's disease, in which I was unwilling to agree with the diagnosis, autopsy revealed that the adrenals were not diseased. I make these statements only to emphasize that the correlation of satisfactory experimental work with clinical studies has afforded a much

better comprehension of this disease than might have been possible without the aid of experimentation upon animals.

The characteristic symptoms of the syndrome described by Addison are "anæmia, general languor and debility, remarkable feebleness of the heart's action, irritability of the stomach, and a peculiar change of colour in the skin." Quoting further from his report:

> "It occurs in both sexes generally, but not exclusively, beyond the middle period of life, and, so far as I at present know, chiefly in persons of somewhat large and bulky frame, and with a strongly-marked tendency to the formation of fat. It makes its approach in so slow and insidious a manner that the patient can hardly fix a date to his earliest feeling of that languor which is shortly to become so extreme. The countenance gets pale, the whites of the eyes become pearly, the general frame flabby rather than wasted; the pulse, perhaps, large, but remarkably soft and compressible, and occasionally with a slight jerk, especially under the slightest excitement; there is an increasing indisposition to exertion, with an uncomfortable feeling of faintness or breathlessness on attempting it; the heart is readily made to palpitate; the whole surface of the body presents a blanched, smooth and waxy appearance; the lips, gums, and tongue seem bloodless; the flabbiness of the solids increases; the appetite fails; extreme languor and faintness supervene, breathlessness and palpitations being produced by the most trifling exertion or emotion; some slight œdema is probably perceived about the ankles; the debility becomes extreme. The patient can no longer rise from his bed; the mind occasionally wanders, he falls into a prostrate and half-torpid state, and at length expires. Nevertheless, to the very last, and after a sickness of, perhaps, several months' duration, the bulkiness of the general frame and the obesity often present a most striking contrast to the failure and exhaustion observable in every other respect."

Up to the present time, little, if anything, has been added to this description of Addison's disease that is helpful in the diagnosis of typical or atypical cases of the malady or in the recognition of the disease in its incipient stages. Diagnosis, when made, has generally depended upon the existence of cutaneous pigmentation together with muscular asthenia, low blood pressure, and sometimes gastric disturbances, as reported by Addison and described in text-

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OF MEDICINE
1930

books. *It should be recognized that this combination of symptoms, when present in a patient as manifestations of adrenal insufficiency, must be regarded as evidence of well advanced disease of the glands.* It is very desirable, therefore, to obtain information concerning the underlying physiological disturbances and the significance of the symptoms, in order to better comprehend the condition and, if possible, to recognize it earlier, also to recognize atypical cases. This I have hoped to facilitate by correlating our experimental work upon animals with clinical observations upon Addisonian patients. The results of years of study can be considered only a beginning in this direction, but the progress made is encouraging and the information obtained affords a better understanding of the pathology of Addison's syndrome, its diagnosis and treatment.

Experience has led me to believe that the statement made by Addison concerning the occurrence of the disease "generally beyond the middle period of life," and "chiefly in persons of a somewhat large and bulky frame", is incorrect. His statement is obviously based upon a number of cases, included in his report, that cannot be considered as cases of adrenal insufficiency. Out of the eleven cases, described by him, not more than six can be admitted to have been genuine Addison's disease, and one of these, a patient over fifty years of age, is doubtful because death may have been due to tuberculosis of the intestines, although both adrenals were also involved. The others were under the middle period of life. Of the five cases which I do not consider as cases of Addison's disease and which constitute the basis for the statement that the disease occurs in later life, four died of cancer and one of tuberculosis. *In all of these five cases only one adrenal was involved.* Contrary

to Addison's suggestion, I have found that the disease occurs much more frequently *before* the middle period of life, and with one exception, I cannot say the cases I have seen occurred in individuals of a large and bulky frame, although extreme emaciation is rather rarely encountered.

I desire to call attention to two symptoms, not heretofore described, one of which I consider quite important because of its relative constancy in human cases as well as in experimental animals, the other perhaps less important because of its probable significance. The first of these symptoms is an *aversion to fatty food.* I have found this symptom present in nearly all cases of Addison's disease that have come under my observation since my attention was attracted to it in our experimental animals. Careful interrogation of patients, avoiding leading questions, often reveals the occurrence of this among the earliest symptoms to develop, together with beginning languor, anorexia or nausea. When muscular and circulatory asthenia, pigmented skin, and gastric irritability are already present aversion to fatty food is a common symptom. The patient may state that a dislike has been developed for milk, or that cream with breakfast cereal is not tolerated, and only lean meats, fish or white meat of chicken can be eaten, whereas previously cream, milk, or fatty meats were eaten without discrimination. This symptom is of considerable interest and I believe of significance, in view of the fact that we have found, almost invariably, a profound congestion of the pancreas at autopsy in doubly adrenalectomised animals, and to a lesser degree in some Addisonian patients. The other symptom mentioned is also quite common. Upon moderate pressure in the costo-lumbar angle a dull ache or pain is elicited especially on the side where at autopsy it was found that the adrenal had

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of Medicine
1930

been undergoing extensive caseous degeneration or suppuration.

As is the case with experimental animals, patients with Addison's disease show little or no significant change in blood chemistry during remissions or periods of improvement under treatment. When a patient is manifestly declining, or during a period which will ultimately lead to an acute exacerbation, as well as during an exacerbation, I have often found an increase in the total NPN, urea N and the undetermined fraction of the NPN, as well as a moderate hypoglycemia and sometimes a moderate hypercalcemia. This, and the gradual onset of symptoms which increase in severity, the character of the symptoms, their resistance to previously employed treatment, and the results of our investigations upon experimental animals, all lead to the conclusion that Addison's disease is the manifestation of a severe intoxication resulting from loss of adrenal cortical or interrenal gland function. This interpretation finds much more support from clinical and experimental evidence than the old idea that Addison's disease is the manifestation of a lack of circulating epinephrin (adrenalin) in the body, and upon it is based the treatment that I have employed with greater success than has been attained by other methods of treatment heretofore recommended.

Treatment

In my earlier experience, patients with Addision's disease who came under my observation were treated by the methods usually employed, but without satisfactory results. Administration of adrenalin and commercial products of adrenal glands proved practically worthless. Indeed, nearly every case that is at present referred to me has been previously treated in this manner. Any supposed bene-

ficial influence from such treatment has been easily explained by the spontaneous improvement which these patients may undergo from time to time. Inasmuch as I am convinced that lack of circulating epinephrin is not responsible, in any important way, for the condition seen in Addison's disease, none of the patients under my treatment receive adrenalin in any form. They are treated entirely on the basis of the results of our experimental work and very encouraging results have been obtained up to the present time.

My treatment consists in the administration by mouth of "Interrenalin", an extract of the interrenal tissue, or cortical portion, of sheep or beef adrenals. This extract is a modified preparation of the product which we found most potent when used intravenously in the treatment of adrenalectomized dogs. In addition to the administration of interrenalin I employ intravenous injections of relatively large amounts of physiological salt solution, or Ringer's solution, to which is usually added about 2 to 6 per cent of dextrose. These injections are employed when a patient manifests the development of intoxication, or during an acute exacerbation. The value of such injections was demonstrated in the prolongation of life and amelioration of symptoms in our adrenalectomised dogs. A number of cases of Addison's disease have been treated with interrenalin alone, and others with the intravenous injections alone. The intravenous injections have been found to be only of temporary benefit and are very useful during an acute exacerbation or in the beginning of treatment of a case, to facilitate elimination of accumulated toxines. Interrenalin gives much more lasting benefit and when employed without the intravenous injections has yielded much more satisfactory

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OF MEDICINE
1930

results than when the injections alone were given. The advantage of supplementing the treatment with interrenalin by intravenous injections of physiological or isotonic solutions when needed is too obvious to require further comment.

In a recent communication[22] I reported some results obtained in a small group of cases by treatment with interrenalin. These results are obviously much better than any hitherto obtained by other forms of treatment in undoubted cases of Addison's disease. Since this report was published I have added a few more cases to my group in which equally promising results have been obtained.

Time will not permit giving details of my records but a very brief abstract from a tabulation will be of interest. Thus, of the six cases that I have mentioned as genuine cases of adrenal insufficiency, in the group reported by Addison, the duration of life from the onset of symptoms was at the longest two years, and in the others from six months to one year. In a group of eight cases that came under my observation before I began treating the disease with interrenalin the duration of life was approximately the same as in Addison's cases. Of six cases that had some treatment with interrenalin, but were obviously in the advanced stages of the disease when treatment was begun, the duration of life in each respective case was as follows: 1½ years, 1 year, 2½ years, 3 years, 2 years and 2 years. In eight cases, in which the diagnosis of Addison's disease cannot be doubted, that are at the present time under my treatment, the duration of the disease since the onset of symptoms has been respectively as follows: 1¾ years, 2 years, 2½ years, 3½ years, 3¼ years, 6 years, 3 years, 1 year. Not only is life being prolonged in these cases of Addison's

disease but the symptoms in most cases are decidedly ameliorated. In some there has been considerable reduction of pigmentation of the skin. The least favourable results have been obtained in blonde individuals, who do not seem to develop the skin pigmentation as readily as individuals of dark complexion when afflicted with Addison's disease. I have been led to consider the prognosis in blondes much more unfavourable, having found that the disease is apparently much more rapidly fatal than in brunettes.

The previously mentioned figures on the duration of the disease in treated and untreated cases can leave no doubt that progress is being made in our knowledge of the physiology and pathology of the adrenal glands, and that treatment of disease of these glands may be more hopeful in the future than in the past. It is scarcely necessary to mention the fact that the result of treatment in a given case of Addison's disease is very largely influenced by the character and degree of the underlying or associated pathological lesions. When there are active or extensive tuberculous processes or malignancy present favourable progress cannot, of course, be expected. Doubtless, also other factors, at present unknown, must influence the results of treatment in some cases.

In conclusion, it is proper to emphasize that the advantages afforded by modern experimental medicine cannot be overestimated, and it is to be hoped that Addison's disease, like other diseases which have hitherto resisted all efforts to combat them, will ultimately yield to appropriate treatment.

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SUPRARENAL CORTICAL EXTRACTS IN SUPRARENAL INSUFFICIENCY (ADDISON'S DISEASE) *

CLINICAL AND EXPERIMENTAL STUDIES ON ADRENAL INSUFFICIENCY AND ADDISON'S DISEASE AND THE TREATMENT OF SUCH CONDITIONS BY INTERRENALIN

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INTRODUCTORY REMARKS BY THE PRESIDENT, DR. H. LISSER

r. Rogoff and Members of the Academy:

Some ten years ago when I became especially interested in Endocri- logy, it was my good fortune to encounter an article by Doctors Stewart d Rogoff, one of those rare papers which arrests one's attention and ves an enduring impression. It dealt with adrenal insufficiency and was markable for its scientific clarity not only, but also for its distinguished tion. The following quotation eloquently conveys the tone of the paper:

> The contrast is great when we leave this desert, where the physiologists and erimental pathologists have wandered, striking many rocks but finding few ings, and pass into the exuberant land of clinical endocrinology, flowing with ndest milk and honey almost suspiciously sweet.

Some years later I was privileged to spend several hours in Dr. goff's laboratories at the Western Reserve University in Cleveland, ere he still continues to prosecute his painstaking investigations. I be- ve it can be fairly stated that the experimental researches of Drs. wart and Rogoff in the domain of adrenal insufficiency constitute the st extensive and convincing work on this subject done anywhere in the rld.

It is therefore in no perfunctory manner that I express my pleasure in roducing Dr. Rogoff, Associate Professor of Experimental Medicine Western Reserve University, Cleveland, whose topic for this evening l be "Clinical and Experimental Studies on Adrenal Insufficiency and dison's Disease and the Treatment of Such Conditions by Interrenalin."

I desire, first of all, to express my keen appreciation for this portunity of coming so far from home and being given the nor to address you on the work which has occupied my atten- n for the past fifteen or sixteen years. I wish to correct what ght be an erroneous impression, in case you have been led to ieve that the words just quoted by your president are mine.

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1930

It so happens that during the past fifteen years I have indee
been "wandering in the desert sands, striking many rocks bu
finding few springs"; I have occasionally found a valuabl
nugget and in the refining process my venerable colleague an
collaborator, Stewart, has contributed the finishing touches wit
his delightful choice of language and charming metaphori
phraseology. Our association and friendship has been so lon
and intimate that I am very often credited with Stewart
virtues, while he is accused of my faults.

Tonight it is my pleasure to present to you some of the re
sults of my work, and in the subject that was chosen, "Clinica
and Experimental Studies on Adrenal Insufficiency an
Addison's Disease, and the Treatment of Such Conditions b
Interrenalin," I shall aim to correlate our fundamental physio
logical investigations with the clinical application of the subjec
in so far as this is possible within scientific bounds. The wor
that has been conducted at our laboratory should interest clinica
men, especially, since here is a subject in which our knowledge o
the physiology of an important organ began with a clinical ob
servation. The studies which followed this clinical observation
however, became so confusing and misleading that physiologist
and clinicians are compelled to re-investigate the entire subje
of adrenal function and insufficiency, and it is with such re
investigation that I have been chiefly occupied for more tha
fifteen years. Our labors have yielded some interesting infor
mation and have led to different concepts, than formerly pre
vailed, of adrenal function in health and in disease. I desire t
emphasize a new conception of Addison's disease, the clinica
condition with which we are familiar as representing the mos
severe form of adrenal insufficiency. Heretofore, and indeed u
to the present time, it has been generally assumed that Addison'
disease is the direct result of lack of a circulating hormone fror
the adrenals. This hormone has been thought to be epinephri
(adrenalin), the secretion from the adrenal medulla. Our wor
has led us to discard this conception in favor of the idea tha
lack of a circulating hormone, alone, does not explain Addison'
Disease and that *if lack of a circulating hormone is the impor*

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May 11, 1929, Vol. 92, pp. 1569-1571

SUPRARENAL CORTICAL EXTRACTS IN
SUPRARENAL INSUFFICIENCY
(ADDISON'S DISEASE) *

nt factor in the disease it is not epinephrin, but "interrenalin," he active product of the adrenal cortex.

When the keen observer, Thomas Addison, selected a group f cases, out of those in which he was making studies on prinary anemia, and found that this particular group of cases difered clinically from the anemias, in certain respects; when he bserved, in all these cases, that there was a pathological condiion in the suprarenal bodies, he laid the foundation for our presnt knowledge and work on the adrenal glands. Within the year ollowing Addison's report, and up to the present time, physilogists have attempted to produce experimental adrenal inufficiency in animals, by excision or destruction of the glands, ut practically always without success. Their animals either died rithin a very short time, as the result of poor surgery, or surived because of incomplete removal of the adrenal cortical tisie. It must be remembered that any fragment of adrenal cortex hich suffices to sustain life will also prevent the development f symptoms of adrenal deficiency. However, we have been able develop a satisfactory technique for surgical removal of both drenals in animals and have accumulated large series of adrenaltomized dogs, cats, guinea pigs, rabbits, rats, and a number of ionkeys, which have survived the operation in excellent health r relatively long periods before the onset of the fatal condition iat is due to loss of adrenal function. This has enabled us to ake studies upon animals with adrenal insufficiency uncomicated by other conditions which would contribute largely, if t entirely to the fatal outcome. In the case of other endocrine ands, extirpation has proven relatively easy compared with lrenalectomy. Even the rather difficult endocrine operation, moval of the pituitary, is perhaps less dangerous than removal the adrenals, in view of the serious physiological consequences iat are associated with this surgical procedure when not perrmed in a very particular and skillful manner. Most of the rlier work on adrenalectomy has led to the proper conclusion at the adrenals are indispensable for life and health. The conision, I may say, was quite correct, but the surgical experiients by which that conclusion was reached could not have

demonstrated that the adrenals are essential for life. Indispensability of the glands was well established only from the original clinical observation, by Thomas Addison, that in cases in which the adrenals had been destroyed, almost completely or completely by disease, death rapidly ensued. Satisfactory experimental evidence, from properly adrenalectomized animals, has become available only within recent years, almost entirely from the results obtained in our laboratory.

About forty years after Addison described the syndrome which bears his name, Oliver and Schäefer discovered that extracts made from the adrenals are capable of markedly elevating the blood-pressure when such extracts are introduced into the circulation of animals. Shortly thereafter the potent principle that is responsible for this blood-pressure effect, adrenalin, was extracted and obtained in pure form. From the facts that in Addison's disease the blood-pressure is decidedly lower than normal, that adrenalin introduced into the circulation is capable of elevating blood-pressure and from absence of knowledge concerning any other substance than adrenalin in the adrenals, it was quite natural to have concluded that Addison's disease is a manifestation of lack of circulating adrenalin. This concept has been and is still, largely or entirely, the basis for treatment, by some excellent clinicians, of Addison's disease and other conditions supposed to be associated with lack of adrenal function. However, in our earlier work we demonstrated conclusively that the serious consequences of adrenal deficiency must be due to interference with cortical (interrenal gland tissue) and not medullary (adrenalin secreting portion) function, since it is possible, in experimental animals, to totally suppress the epinephrin secretion without causing harmful results. This is in harmony with certain clinical observations that have been made by others as well as by myself, that in some cases of Addison's disease nothing pathological is found in the medulla of the adrenal, the disease being limited to the cortex.

Experimentally, it is possible to remove completely one adrenal gland, three-quarters or seven-eighths of the remaining gland, cauterize or curette the portion of the remaining cortex

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May 11, 1929, Vol. 92, pp. 1569-1571

SUPRARENAL CORTICAL EXTRACTS IN SUPRARENAL INSUFFICIENCY (ADDISON'S DISEASE) *

vhich was in contact with the medulla (or cut out a wedge-shaped piece, curetting away any portion of the medulla that night have been left), and, inasmuch as epinephrin is not secreted from the adrenal in the absence of its nerve supply, as in additional precaution the remaining fragment of cortex can be completely denervated. This is accomplished by section of the major and minor splanchnic nerves, extirpation of three or four of the lumbar sympathetic ganglia just below the diaphragm, and extirpation of the celiac ganglion. An animal possessing only a fragment of cortex, and that completely denervated, will ive indefinitely in excellent health and continue to perform all he functions and usual habits of animal life in the laboratory. But, if now we remove a portion, or the balance, of the remaining cortex the result is just as if we had removed both adrenals, presenting the characteristic symptoms of acute adrenal insufficiency followed by death within the usual period. In rabbits r rats it is possible to extirpate both adrenals without harmful esults in a large proportion of the animals. This is because such nimals possess accessory cortical bodies. The epinephrin secretion from the adrenals has been totally suppressed by removal f the glands, yet life and good health are not affected so long as here is sufficient *interrenal tissue* present. In certain fishes the wo portions of the gland exist as separate bodies—the chromophil material, corresponding to the medulla, and the interrenal ody, corresponding to the cortex of the mammalian adrenal land. If the interrenal body is removed death ensues, but destruction of the chromophil tissue is not incompatible with life. Thus, it is evident that the cortex and not the medulla of the drenal gland is indispensable; that removal of both adrenals auses death, not because of suppressed epinephrin secretion but ecause of loss of a function that resides in the cortex of the land.

The question may be asked whether the function of the interrenal tissue consists of elaboration and secretion of an indispensable hormone, the lack of which, in the circulation, gives se to the condition known as Addison's disease or whether the drenal cortex serves as a detoxicating organ, removing from

the circulation certain harmful substances which if present in excess give rise to the disease. In short, is Addison's disease directly due to the absence of an essential cortical hormone in the blood, or to the presence of toxic material in the circulation which the adrenal cortex is normally capable of removing or neutralizing? My clinical as well as my experimental studies have yielded evidence that Addison's disease and experimental adrenal insufficiency are manifestations of an intoxication, and that all of the symptoms, including muscular and circulatory asthenia, low blood-pressure, gastro-intestinal disturbances, and even the pigmentation, are evidences of an accumulation of certain toxins, probably specific for this particular type of deficiency or syndrome. The symptoms may not necessarily be direct manifestations of absence of a specific hormone from the circulation, but the intoxication undoubtedly results from lack of function of a specific substance elaborated by the adrenal cortex, for which we have suggested the name "interrenalin," indicating its origin in the interrenal gland tissue.

The results of practically all attempts to create adrenal insufficiency in animals, by surgical removal of the glands, from the first experiments by Brown-Sequard, in 1856, up to the present time, might lead to the conclusion that death following excision of the adrenals is the direct result of acute suppression of a very potent internal secretion, since animals did not survive adrenalectomy long enough to warrant the suggestion that the symptoms and fatal outcome might be the consequence of an intoxication. I have already indicated that, although the conclusion that the adrenal glands are indispensable for life has prevailed from the earliest experiments, it may be considered merely as a fortunate guess since the results of the adrenalectomies were much too poor to yield reliable information. I do not hesitate to apply this same statement to many of the alleged physiological phenomena described in studies on the adrenal glands that have appeared in recent literature. For, only too often is it possible to detect, in the report of a research, evidence of inadequate technique, unsatisfactory methods, or unsound interpretation. This is not encouraging, for scientific progress,

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May 11, 1929, Vol. 92, pp. 1569-1571

SUPRARENAL CORTICAL EXTRACTS IN SUPRARENAL INSUFFICIENCY (ADDISON'S DISEASE) *

but is perhaps to be expected in the plethora of literature that results from the modern race for publication.

Brown-Sequard adrenalectomized rabbits, rats, guinea pigs, cats, and dogs. All the animals died in 9 to 37 hours. Even after excision of only one gland his animals died within 24 hours. Such animals could yield no useful information, and there can be no doubt that the poor results were due to inadequate surgical technique; but in making this criticism I must remark that it is amazing to find that, with all the advantages of modern surgery, most of the more recent workers obtain results that are no better than those obtained in 1856. Biedl, in his book on the internal secretions, quotes the work of Strehl and Weiss as representing the best results on adrenalectomy. Although their results are much better than were obtained by most of the earlier workers and correspond well with some of the better results reported in quite recent literature, it will be obvious when I present some of our results, later, by lantern slides that, at best, they only demonstrate the relative tolerance to shock and surgical insult among dogs, cats, rats, rabbits, and guinea pigs. The survival period of their adrenalectomized rabbits was 8 to 76 hours, rats 5 to 19 hours, and guinea pigs 4 to 9 hours. In my experience, guinea pigs survive an average of about a week and it is well known that a considerable proportion of rats and rabbits will survive indefinitely, if not killed by poor surgery, since accessory cortical bodies exist in a large proportion of these animals. It is rather distasteful to constantly engage in destructive criticism and I would much prefer to avoid it. However, since the bulk of the literature on the subject is based upon work of inferior quality, it is essential to destroy erroneous concepts, if progress is to be made and constructive effort recognized. In our studies we have devoted years in acquiring experience and developing suitable technique and methods to enable us to obtain useful information. We have never been so fortunate as to make important discoveries over night or to get the best results obtainable without practice and much labor. Occasionally, it might be suspected that some investigators are more favored by fortune than we, in this respect.

The animal of choice for studies on adrenal insufficiency is the dog. In this animal, existence of accessory adrenals is rare, and "proper" adrenalectomy always leads to a fatal outcome preceded by a train of characteristic symptoms. The superior intelligence of the dog over other laboratory animals facilitates clinical studies, and its size permits withdrawal of satisfactory specimens of blood for examination, without introducing a serious factor in the survival of the animal. Other investigators have been unable to obtain survival of adrenalectomized dogs beyond a few hours up to a day or two, among the best results up to 4 or 5 days. Table 1 is a reproduction of a table from Paper V of our "studies on adrenal insufficiency." It demonstrates that the average period of survival of our untreated adrenalectomized dogs is about 10 days, the minimum in the neighborhood of a week and the maximum about two weeks. This permits studies to be made during a relatively long period of good health after loss of the adrenals, as well as after development of the symptoms which lead to death. For testing the potency of adrenal extracts I employ only male dogs as controls, since we have demonstrated that pregnancy or rut can prolong life in adrenalectomized animals. The protective influence of pregnancy is demonstrated in Table 2 (p. 16), which is a reproduction of a table in Paper III of our series.

Cats have been employed by some investigators for studies on adrenal deficiency. The surgical operation is much easier than in dogs, but, for reasons which I shall state, the cat cannot yield as reliable information as the dog. The best results on adrenalectomy in cats obtained by other workers yield an average survival period of a little over 5 days. Our results, recently published in the *American Journal of Physiology,* demonstrate that untreated adrenalectomized cats survive an average of 11 to 12 days, a number of animals living as long as 5 to 6 weeks. In this series, accessory bodies, when found, were removed at the time of the operation for excision of the adrenals. Approximately 8 per cent of the animals possessed accessory adrenal bodies. Had these bodies been overlooked, a number of the cats would undoubtedly have survived relatively long periods, if not

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May 11, 1929, Vol. 92, pp. 1569-1571

SUPRARENAL CORTICAL EXTRACTS IN SUPRARENAL INSUFFICIENCY (ADDISON'S DISEASE) *

ıdefinitely. It is obvious, therefore, that studies concerning the urvival of adrenalectomized cats might yield misleading inforıation, even in the hands of an investigator properly qualified to erform the surgical operation.

TABLE 1

Additional Control Adrenalectomized Dogs

Number of Animal	Weight: First Operation	Weight: Second Operation	Days between Operations	Survived		Began to Refuse Food, Days	Alimentary Canal: Blood	Alimentary Canal: Congestion	Pancreas Congestion
	kgm.	kgm.		da.	hrs.				
-4......	9.1	9.1	11	11¾		10	+++	+++	++++
-6......	5.8	6.5	23	8½		7	++	+	+
-8......	7.4	7.3	41	8½		6			
-9......	9.2	8.3	53	15¾		14	++++	++++	++++
-0......	11.1	9.7	53	6½		5	+++	+++	++++
-1......	7.9	7.8	14	11	5½	10	0	0	++
-2*.....	11.7	9.3	56	5	4½	4	++	++	+++
-3......	10.0	10.0	40	12½		11	++	++	+++
-0......	11.3	11.2	20	9	7	8	+++	++	++++
-7......	10.9	8.5	42	12½		10	0	0	+++
-2......	12.2	11.7	15	14⅔		13	0	+	+
-9......	9.1	9.8	37	15	17	12	++	+++	+
-4......	8.6	10.6	35	4	20	4½	+	+	+++
-8......	9.4	10.8	29	11	23	10	+++	+++	+++
-9......	7.0	7.5	50	8	22	7	+	0	++
-0......	9.6	9.9	32	6	4½	5	++	++	++++
1-1......	7.1	9.6	55	8	23	7	++++	++++	++++
1-2......	6.9	8.7	55	9½		7	++++	++++	++++
1-3......	7.2	7.9	16	8¾		7	+++	+++	++
1-4......	6.3	7.0	30	6⅓		4	0	+	+++
1-8......	6.2	6.1	19	6½		3-4	0	+	++
1-9......	11.4	12.1	21	15	17¾	13	+++	+++	++
1-0......	9.9	10.4	21	11			0	0	+++
1-1†.....	9.3	9.9	16	4	20	3	0	0	++
1-3......	13.0	13.0	16	13½		12	+	+	++++
1-3......	9.1	11.0	32	4	2½	3	++	++	++++
1-4......	9.5	8.2	18	5	9	4	+	++	++++
1-5......	5.7	5.8	15	11	15	9	+++	++	++++
1-6......	7.5	8.2	30	12	16	10		+	+++
1-2......	9.3	9.2	21	12	6½	11	++++	++++	++++
1-5......	9.5	9.2	15	12	4		+++	+	++++
1-2......	6.7	6.4	13	5	8	4		++	++++
1-4......	8.7	8.1	13	7	10½	5	+++	+++	++
1-0......	13.1	13.1	20	9½		8	+++	+	+++
1-4......	14.5	14.0	15	6⅚		5	++	+++	++++
1-6......	7.5	8.1	15	9	3½	7	+++	+++	+++

* Had weakness and tremors in muscles of legs and neck before and after erations.

† Very mangy before and after operations.

When skilfully done, adrenalectomy in all laboratory animals is followed by rapid post-operative recovery. Our dogs are in excellent condition 10 or 15 minutes after the operation and would readily take food within a half-hour, if permitted. They continue in excellent health, indistinguishable from normal, un-

TABLE 2

ADRENALECTOMIZED PREGNANT DOGS*

Number of Dog	Weight at		Days between Operations	Survived		Began to Refuse Food, Days	Adrenal Weights		Alimentary Canal		Pancreas Congestion
	First Operation	Second Operation					Right	Left	Blood	Congestion	
	kgm.	kgm.		da.	hrs.		g.	g.			
996..	8.0	8.7	19	15	2	12½	0.63	0.78	+++	+++	+++
1002..	11.6	13.3	13	32	16½	29	0.78	0.90	++	++	++
1026..	11.5	13.3	39	17	6	14½	0.75	0.85	+++	+++	+++
1034..	8.3	8.9	8	26	18½	26	....	....	+	0	0
1036..	9.9	9.5	9	46	3½	44	0.76	0.67	+	+++	++++
1128..	13.7	15.1	35	9	11	8½	0.90	0.72	+	+	+++
1137..	10.9	11.7	31	1	7	1	0.75	0.70	+	+	+++
1143..	10.4	11.2	23	25	5	24	0.85	0.92	+	+	+++
1157..	8.7	11.9	177	57½		57	0.57	0.78	+	+	0
1162..	9.4	11.6	127	13	7½	12½	0.75	0.90	++++	++++	++++
1168..	10.4	11.0	10	2	18	2	0.55	0.67	+	0	+++
1174..	6.2	8.7	120	58	3	55	0.40	0.50	+	0	++
1176..	11.6	12.6	79	14⅔		10½	0.75	....	+++	+++	++++
1179..	15.7	17.3	50	12¾		11½	1.02	0.80	+++	+++	+++
1185..	10.3	11.4	30	14	3½	12	0.64	0.70	++	++	++++
1192..	9.7	10.4	19	22⅜		19½	0.80	....	+	++	+++
1198..	11.2	11.4	11	6¼		3½	....	0.85	++++	++++	++++

* The right adrenal was excised first in all the dogs except 1034, 1036, 1128, and 1137.

operated dogs, until about one to three days preceding death. The blood-pressure remains unaltered, appetite and digestion excellent, no change from normal in blood chemistry, and the animal is fully capable of defending himself in combat with other dogs. Blood-pressure can be satisfactorily measured, in unanesthetized dogs, by means of a sphygmomanometer, with a small cuff devised to fit around a loop previously prepared by displacing a portion of the carotid artery within a flap of the skin of the neck. Table 3 illustrates the maintenance of normal blood-

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SUPRARENAL CORTICAL EXTRACTS IN SUPRARENAL INSUFFICIENCY (ADDISON'S DISEASE) *

TABLE 3

BLOOD-PRESSURE OBSERVATIONS, DOG

Date	Time	Blood-Pressure						Pulse Rate per Minute
		Systolic			Diastolic			
		Maximum	Minimum	Average	Maximum	Minimum	Average	
		mm.	mm.	mm.	mm.	mm.	mm.	
May 11	10:00 a.m.	162	150	155.0	110	98	103.4	160
May 19	4:00 p.m.	162	152	156.9	112	93	103.5	128
May 20	5:30 p.m.	179	150	164.5	100	93	96.5	160
May 23	1:00 p.m.	152	138	142.5	98	88	92.1	144
May 26	4:30 p.m.	153	148	149.9	103	96	100.1	104
June 9	4:00 p.m.	145	135	139.2	105	92	97.3	144
June 10	5:00 p.m.	145	128	136.5	90	84	88.3	136
June 12	2:00 p.m.	150	139	143.9	105	95	97.2	144
June 14	4:20 p.m.	129	124	125.8	86	75	79.7	136
June 15	2:15 p.m.	Right Adrenal Extirpated						
	4:15 p.m.	175	174	174.2	125	123	124.0	124
June 16	10:00 a.m.	158	142	150.0	110	100	103.9	120
	5:40 p.m.	162	155	158.0	106	102	105.4	104
June 17	10:00 a.m.	156	152	154.5	111	110	110.1	88
	5:20 p.m.	147	140	142.8	105	98	101.0	86
June 18	10:40 a.m.	145	140	142.4	105	92	97.7	88
July 29	4:45 p.m.	146	144	144.9	112	100	103.3	152
July 30	5:36 p.m.	146	139	142.0	110	102	104.4	120
July 31	10:42 a.m.	Left Adrenal Extirpated						
	4:40 p.m.	170	162	167.3	130	130	130.0	112
August 1	2:20 p.m.	148	142	143.6	120	115	117.0	104
August 2	10:15 a.m.	162	158	160.8	135	120	124.1	108
	5:20 p.m.	150	142	145.0	100	92	96.2	88
August 3	9:55 a.m.	153	146	149.2	117	110	113.0	96
	5:30 p.m.	152	140	147.6	130	115	118.0	104
August 4	10:15 a.m.	140	120	131.1	110	90	100.8	128
	5:20 p.m.	148	140	142.9	110	94	102.7	112
August 5	10:15 a.m.	140	122	132.8	110	90	98.3	128
	5:15 p.m.	132	124	129.9	104	94	98.9	128
August 6	10:30 a.m.	121	102	112.6	87	76	82.9	128
	4:35 p.m.	110	95	101.8	82	78	80.6	152
August 7	10:40 a.m.	98	76	87.0	70	58	63.5	140
	4:10 p.m.	92	75	85.0	70	54	61.1	148
August 8	12:30 p.m.	100	92	95.2	68	60	63.5	120
August 9	4:35 p.m.	103	88	99.8	..	..	....	136
August 10	9:40 a.m.	89	70	79.2	..	..	....	136
	4:20 p.m.	90	78	84.1	53	50	51.0	128
August 11	10:00 a.m.	98	88	91.5	..	..	....	124
	3:50 p.m.	77	70	72.7	..	..	....	120

pressure until the onset of symptoms, in a dog who survived 12 days following extirpation of the second adrenal. Chart I is a graphic representation of the maximum and minimum systolic and diastolic pressures observed in a dog before adrenalectomy, after unilateral adrenalectomy, and after excision of the remaining gland. The animal was in rut at the time of the second adrenalectomy and survived complete loss of the adrenals for 36 days. Normal blood-pressure was maintained until about 4 days preceding death, when the symptoms of adrenal insufficiency developed. Similar results were obtained with adrenalectomized rabbits, regardless of the duration of survival. It may be remarked that, although accessory cortical bodies were responsible for indefinite survival in a number of rabbits, the animals had been deprived of the adrenal medulla by double extirpation of the glands. It is obvious, therefore, that absence of the epinephrin secretion did not influence the maintenance of normal blood-pressure.

Almost invariably, the first symptom that occurs in animals deprived of their adrenals is anorexia, which develops about 2 to 4 days before the fatal outcome. At first the animal has an aversion to fatty food, but will eat lean meat and other food. The loss of appetite then becomes complete and gastro-intestinal disturbances develop, including bilious vomiting and diarrhoea, often with bloody stools. Muscular and circulatory asthenia follows, gradually becoming more pronounced within a day or two, and quite commonly symptoms of disturbances in the nervous system are present. These consist of hallucinations, yelling and racing fits, muscular twitching, and tetanic convulsions, with coma followed by death of the animal.

Shortly preceding, or with the onset of the symptoms, there is an increase in the total non-protein nitrogen of the blood, the urea nitrogen and the so-called undetermined fraction of the non-protein nitrogen. There is no significant change in the amount of creatin, creatinine, uric acid, or amino-acid nitrogen. The blood-sugar level is usually somewhat diminished but not to such an extent as to suggest any relationship between the hypoglycemia and the convulsions. The hypoglycemia rarely exceeds

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May 11, 1929, Vol. 92, pp. 1569-1571

SUPRARENAL CORTICAL EXTRACTS IN SUPRARENAL INSUFFICIENCY (ADDISON'S DISEASE) *

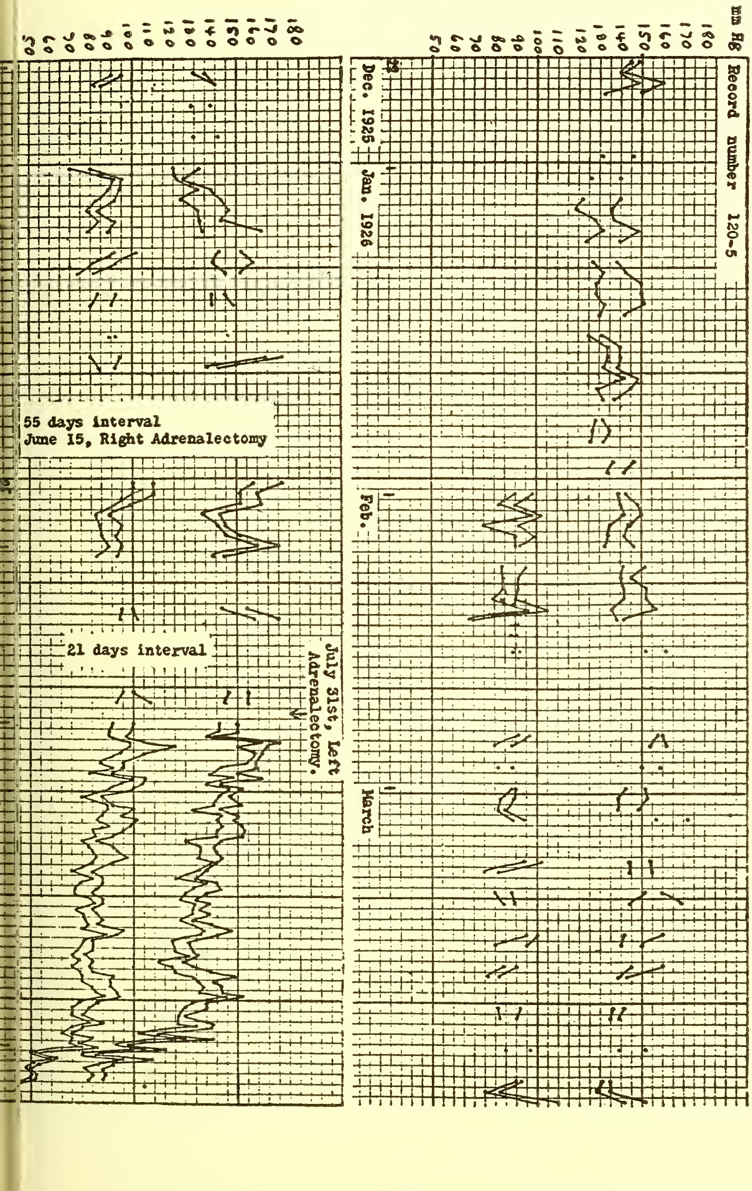

CHART I

Blood-Pressure of Adrenalectomized Dog

the lower limit of the normal blood-sugar level. Chlorides are frequently diminished and blood calcium increased. No significant change is found in the blood cholesterol. Concentration of the blood occurs, often shortly preceding the onset of symptoms, increasing till death. There is an increase in solids, specific gravity, percentage of hemoglobin, erythrocyte count, and in the relative volume of cells. The relative volume of serum is diminished but its content of protein and its specific gravity are unaffected. Table 4, reproduced from Paper III, illustrates that

TABLE 4

Blood Analysis; Adrenalectomized Dogs

Number of Dog	Blood Taken, Date	Previous Meal	Non-Protein Nitrogen	Urea Nitrogen	Uric Acid	Creatinine Preformed	Creatinine Total	Amino-Acid Nitrogen	Undetermined Fraction	Dextrose	Cl as NaCl
1174	5-6	Meat	43	26	1.3	1.0	2.3	9.3	6.5	....	528
	5-9	Bread, milk....	27	11.1	1.4	1.0	2.8	10.2	4.3	....	495
	5-11	Meat	36	21.5	1.2	0.9	2.3	8.9	4.4	....	520
	5-14	Bread, milk....	28.4	14.7	1.3	0.9	2.5	10.0	2.4	....	520
	5-20	Meat	46	25	1.2	1.0	2.4	9.4	10.4	0.08	520
	6-8	Meat	42.8	22	...	1.0	3.3	9.6	9.7	0.08	503
	6-14	Bread, milk....	38	23.4	1.6	1.3	3.0	8.8	4.3	0.08	510
	6-28	Meat	48	37.5	1.4	1.0	2.4	8.8	0.4	0.07	553
	6-30	Rabbit	72.3	48.4	1.1	1.1	2.5	9.5	13.2	0.06	570
	7-1	Rabbit	42.8	24	1.2	1.0	2.1	8.9	8.8	0.08	560
	7-2	Rabbit	52.2	38.4	1.2	...	2.2	...	....	0.09	570
	7-3	Not eating.....	82	46.3	1.3	1.1	2.1	9.6	25	0.06	585
	7-4	Not eating.....	156	104	1.2	2.0	5.2	8.2	41.7	0.08	570
1198	4-22	Meat	38	19	1.5	1.1	3.0	13.3	4.2	....	510
	5-6	Meat	39	22	1.7	1.1	2.3	10.3	5.4	....	530
	5-9	Milk, bread....	68	47	1.7	1.9	5.0	10.0	8.8	....	478
	5-11	Meat	60	37	1.7	1.2	2.7	10.0	11.5	....	410
	5-12	Not eating.....	86	52	2.0	1.5	3.7	11.6	20.0	....	430
	5-13	Not eating.....	146	92	3.1	2.3	6.5	13.0	37.5	....	430

the changes in the blood occur at the time that other symptoms of adrenal insufficiency are developing. This table gives the results obtained in two pregnant adrenalectomized dogs, which

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May 11, 1929, Vol. 92, pp. 1569-1571

SUPRARENAL CORTICAL EXTRACTS IN SUPRARENAL INSUFFICIENCY (ADDISON'S DISEASE) *

explains the relatively long survival periods. In non-pregnant animals, however, the same changes occur in the blood, at the same time preceding the fatal outcome.

Post-mortem examination of untreated animals that succumbed as the result of removal of both adrenals generally presents a striking pathological state of the entire alimentary canal. In the stomach contents there is usually found a considerable amount of bile and frequently also blood. Quite commonly the entire small intestine is filled with bloody material. The mucus membrane of the alimentary canal is very much congested throughout, and in some places profoundly hemorrhagic. Ulcers are often present in the stomach and sometimes in the duodenum. The pancreas is almost invariably exceedingly congested. Other organs show no significant gross changes.

The clinical condition, manifested by the symptoms, the blood-changes, and the evidences found at autopsy support the conclusion that loss of the adrenals is followed by development of a very severe and rapidly fatal intoxication; yet, during a relatively long period, which precedes the onset of symptoms, nothing abnormal can be detected in the animal. Since total suppression of the epinephrin secretion does not cause any harmful influence upon the animal, the fatal outcome of removal of the adrenals and the symptoms of the severe intoxication which precedes it can only be due to loss of the cortical or interrenal gland tissue. It is highly probable that the loss of a specific hormone (interrenalin) which is essential for normal metabolic activity is responsible for the development of a severe intoxication. That the symptoms are directly due to acute suppression of secretion of this hormone is less likely, since if this were the case it would be expected that the symptoms would develop very shortly after depriving the animal of its adrenals. It might be argued that during the period of good health of the animal there still exists a residue of the hormone in the circulation or that perhaps some other organ vicariously functions, during this period, in place of the adrenals. Indeed, the latter idea is suggested by our observation that the condition of rut or pregnancy exercises a protective influence in adrenalectomized dogs. The

former is less likely from the fact that the beneficial influence of interrenalin is not immediate but requires repeated and continued administration.

Additional convincing evidence, which supports my view that adrenal insufficiency, or Addison's disease, essentially represents an intoxication rather than acute suppression of a circulating hormone, is afforded by our demonstration that life can be considerably prolonged, in completely adrenalectomized animals, by intravenous administration of relatively large volumes of simple physiological salt solutions. This is illustrated in Table 5, reproduced from Paper IV. Such treatment obviously cannot supply a missing hormone. The beneficial influence can only be due to dilution and elimination ("washing out") of toxic material,

TABLE 5

ADRENALECTOMIZED DOGS* TREATED BY INTRAVENOUS INJECTIONS OF RINGER'S SOLUTION

Record Number	Weight		Interval between Operations	Survived		Began to Refuse Food, Days	Adrenal Weights		Alimentary Canal		Pancreas Congestion
	First Operation	Second Operation					Right	Left	Blood	Congestion	
	kgm.	kgm.	days	da.	hrs.		g.	g.			
86-2..	...	....	12	19	12	8	....	....	+		+
96-6..	9.5	10.2	6	19	8	10	0.60	0.65	+—	+—	
97-8..	11.7	12.9	27	33	5	16	0.62	0.56	+++	+++	+++
98-0..	11.5	11.0	12	14	20	13½	0.65	0.54	++	++	++
100-2..	11.6	13.3	13	32	17	26	0.78	0.90	++	++	++
100-5..	8.1	8.3	12	15	5	11	0.45	0.45	+	+	++++
102-6..	11.5	13.3	39	17	6	14½	0.75	0.85	+++	+++	+++
113-1..	9.2	8.5	31	6	16	5	0.40	0.37	++	++	++++
113-3..	10.2	9.3	31	5	12	5	0.57	0.66	0	+	+++
113-4..	9.4	9.5	53	14	4	4½	0.58	0.48	+—	0	++
114-0..	12.4	12.6	14	13	15	7	0.70	0.60	++	+	++
117-0..	11.1	10.3	33	53	16	45	0.70	0.60	+—	0	++++
117-2..	7.7	7.5	33	38	2	19	0.65	0.72	+	+	++++
118-3..	11.2	10.8	18	20		19	0.55	0.60	++	+++	++++
118-4..	6.8	7.5	30	20	3	15	0.65	0.80	++	++	+
119-0..	10.7	10.8	23	3		3	0.73	0.85	+	++	++++
119-1..	9.1	8.8	23	6	12	6	0.72	0.86	0	0	+++

* All dogs are males except 100-2, 102-6, 113-4, 118-3, and 118-4. Right adrenal excised first in all the dogs except in 113-1, 113-3, 113-4, and 114-0.

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May 11, 1929, Vol. 92, pp. 1569-1571

SUPRARENAL CORTICAL EXTRACTS IN SUPRARENAL INSUFFICIENCY (ADDISON'S DISEASE) *

PLATE I

A

B

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May 11, 1929, Vol. 92, pp. 1569-1571

SUPRARENAL CORTICAL EXTRACTS IN SUPRARENAL INSUFFICIENCY (ADDISON'S DISEASE) *

probably also the increase in circulating liquid affording some relief to the embarrassed circulation. The effect of intravenous administration of Ringer's solution upon the intoxication associated with adrenal insufficiency is illustrated in Plate I. The dog, shown in this figure, developed severe convulsions and coma on the fourth day following complete adrenalectomy, having received no treatment. The chemical changes in the blood, previously described, were present. Plate I, *A,* shows the animal shortly after a tetanic convulsion. He was profoundly asthenic and could not be aroused from the comatose state. An injection of Ringer's solution failed to give immediate relief, although in other animals the immediate improvement is usually remarkable. But the treatment caused considerable diuresis and improved the circulation. Another injection, given a few hours later, again caused diuresis but did not relieve the coma. It appeared that the animal could not be rescued, and as the last injection was given late in the evening the dog was left in his cage for the night, with the expectation that he would be found dead in the morning. To our great surprise, on the following morning, the animal was found quite alert, walking about, apparently in very good condition. Plate I, *B,* illustrates his condition when photographed later in the day. Intravenous Ringer's solution injections were given daily, and the animal continued in excellent health for about a month, surviving into the 34th day following total adrenalectomy. During the period of good health, results of chemical blood analyses were normal.

I have demonstrated that it is possible to extract from the adrenal cortex a substance which is capable of prolonging life of completely adrenalectomized dogs well beyond the maximum period of survival obtained in our large series of untreated, similarly operated, control animals. It is also capable of relieving symptoms of adrenal insufficiency in such animals, often when the condition is so severe that life cannot be prolonged. The results illustrated in Table 6 (p. 24), reproduced from Paper V of our series, were obtained with various extracts prepared from dogs' adrenals, and are similar to the results obtained by us with preparations from slaughterhouse material (beef and

sheep glands). I have obtained potent extracts by extraction with aqueous, alcoholic, and other solvents, in neutral, slightly acid, or slightly alkaline media. The potency of various preparations

TABLE 6

ANIMALS TREATED WITH EXTRACTS OF DOGS' ADRENALS*

Number of Animal	Weight		Days between Operations	Survived		Began to Refuse Food, Days	Alimentary Canal		Pancreas Congestion	Number of Extract
	First Operation	Second Operation					Blood	Congestion		
	kgm.	kgm.		da.	hrs.					
123-0....	9.0	9.0	12	13	13	13	++	++	+++	II
123-1....	7.4	7.7	13	21⅔			0	++	+++	II, V
123-3....	10.0	9.7	13	17⅔		17	0	0	++++	II, V
123-6....	9.0	8.8	15	9	6	9	+++	++	++	V
123-7....	9.6	9.2	22	10¾		9	++	++	++++	V
123-9....	6.5	6.2	24	6	21½	5	++	+++	+++	V
124-1....	10.6	10.4	21	78⅔		76½	0	0	0	V, XII
124-2....	10.6	9.7	24	6	22	5	++	++	++	VI
124-5....	8.5	9.4	69	12	2	11	0	++	++++	XV
124-7....	9.5	9.6	64	9	4	8	++++	+++	+++	XV
124-9....	9.3	9.5	12	10½		10	++	++	+++	XII
125-2....	8.6	8.4	11	7½		6½	+	+	++++	XII
125-3....	7.8	7.3	13	5⅔			+—	+—	+	XVI
125-5....	10.0	9.6	35	10	23	9½	+++	+++	++++	XIII
125-7....	7.0	7.2	38	27⅔		27	0	+—	+++	XVI
126-1....	13.5	12.2	41	8	21¼		++++	++++	+	XIV
126-6....	10.8	10.2	42	9⅔		8	++	++	+++	XV
126-7....	11.1	10.2	19	7	1½	6	+++	++++	++++	XIV
126-8....	10.9	10.2	20	12⅔		11	+++	+++	++++	XV
126-9....	9.7	9.3	20	12	½	11	0	+	++++	XV
127-3....	12.1	11.3	14	11	4	9½	+++	+++	+++	XVII
127-4....	8.9	8.1	15	7⅔		7	+++	+++	+++	XII†
127-6....	10.9	10.2	17	15	22	15	++	++	+++	XVIII
127-9....	10.8	10.2	19	19¾		19	+++	++	++++	XIX
128-0....	11.8	11.3	18	22⅔		21½	++	++	++++	XIX
128-3....	7.8	7.4	13	9½		7	+++	++	++++	XIX
129-1....	15.3	15.5	16	11⅔		10½	+++	+++	++	XXI
129-3....	14.0	14.6	14	7⅔		4	+++	+++	+++	XXI
129-7....	11.8	11.4	13	5	20	4	++++	+++	+++	XXI

* All the dogs were males except 123-6, 123-9, 124-2, 124-5, 124-7, 125-3, 125-7, 126-6, and 127-6.

† Old material.

often varies though made by the same method. This is probably chiefly due to adsorption of the active material in the process of removal of proteins and other material from the extracts, for purification and concentration.

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May 11, 1929, Vol. 92, pp. 1569-1571

SUPRARENAL CORTICAL EXTRACTS IN SUPRARENAL INSUFFICIENCY (ADDISON'S DISEASE) *

During the past six years I have repeatedly demonstrated, to various groups of clinical and laboratory colleagues who visited my laboratory, a large number of my experimental animals including many adrenalectomized dogs, whose lives were prolonged by the means just discussed. I have also shown a relatively large number of cases of Addison's disease in which unquestionable benefit has been derived from treatment with interrenalin. Results of these experimental and clinical investigations have been published in a series of papers appearing since 1926, entitled *Studies on Adrenal Insufficiency,* and the work is still in progress. Despite a tremendous amount of labor, however, the information that we have obtained, though very interesting and important, can be considered no more than a beginning in the process of unfolding the secrets of the physiology and pathology of the adrenal glands. We can, at present, only claim to have clearly demonstrated that *the indispensable function of the adrenal bodies is performed by the cortex or interrenal gland tissue in the elaboration of a hormone, which we have termed "interrenalin," the lack of which leads to metabolic disturbances that result in the development of a fatal intoxication. Further, that certain extracts, prepared from the cortical portion of adrenals, are capable of substituting, to a certain extent, for the lack of interrenalin in experimental adrenal insufficiency and clinically in Addison's disease.*

Since the publication of our work, there have appeared a number of papers by other writers, alleging to have extracted the active product from the adrenal cortex or to have isolated the "cortical hormone." Time does not permit me to indulge in extensive discussion of any of these papers, but a few pertinent remarks will not be out of place. While some writers appear to be ignorant of the literature on the subject, making no mention or comment of previous work, others seem unable to find accessible literature or information, since they complain that we have not reported our method of preparing extracts, although mentioned in our published papers and discussed by me at meetings of the American Physiological Society, the Thirteenth International Physiological Congress, and other societies. Any advance-

ment over the results obtained in our laboratory would be gratifying, as compensation for our years of effort in building the foundation for studies in this field. But I find it difficult to accept alleged results of work that cannot be said to be based upon well-controlled, properly performed, fundamental physiological experiments. It may be remarked, also, that apparent persistent attempts to encourage, by repeated publication, the use of physiological misnomers, as names for a hormone that has not as yet been isolated or identified, can contribute nothing of value to scientific progress.

At present, the only reliable means available for determining the existence of the active product of adrenal cortex, in an extract of the gland, consists of its potency as a therapeutic substitute for lack of the indispensable adrenal function, in completely adrenalectomized animals. For reasons that I have previously discussed, the dog is the most satisfactory animal to employ. Reference to the literature, covering a period of three-quarters of a century, will render it wholly unnecessary for me to emphasize that, first of all, an investigator must demonstrate that he has gained sufficient experience and has developed adequate surgical skill to enable him to prepare *satisfactory test animals as well as a sufficiently large series of proper controls.*

I have demonstrated that by supplying interrenalin, with intravenous injections of extracts prepared from cortical adrenal tissue, it is possible to retard the progress of the metabolic changes which lead to the severe intoxication in completely adrenalectomized dogs, so that life in such animals can be materially prolonged. The best results have been obtained when administration of interrenalin was begun soon after the animal was deprived of its adrenal glands. As a rule, I have begun the injections of cortical extracts within 24 hours after adrenalectomy, giving 0.5 to 2 cc. of extract once or twice daily. Plate II illustrates the beneficial influence of such treatment. Plate II, *A,* shows a dog, in excellent condition, 32 days after complete adrenalectomy, having received daily injections of adrenal cortical extract. On the 36th day the animal developed convulsions and coma (Plate II, *B*). Intravenous administration of Ringer's

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SUPRARENAL CORTICAL EXTRACTS IN SUPRARENAL INSUFFICIENCY (ADDISON'S DISEASE) *

PLATE II

B

C

A

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May 11, 1929, Vol. 92, pp. 1569-1571

SUPRARENAL CORTICAL EXTRACTS IN SUPRARENAL INSUFFICIENCY (ADDISON'S DISEASE) *

olution was resorted to and followed by recovery of conscious-ıess and cessation of convulsions. Plate II, *C,* shows the animal n good condition on the following day. The injections of Ring-r's fluid were continued daily, together with administration of he cortical extract and the animal survived into the 43d day, fol-owing total adrenalectomy. If treatment with cortical extracts s delayed until the evidences of adrenal insufficiency become pparent, the beneficial influence of interrenalin is less pro-ounced, but symptoms are usually ameliorated and life may be rolonged. Doubtless, much depends upon the severity of the ntoxication and the ability of the animal to eliminate the ac-umulated poisonous metabolites. When a comparatively severe tate of adrenal insufficiency exists, I have found it possible to escue a moribund animal by first relieving the intoxication with ntravenous injections of physiological saline solution and then ontinuing the treatment with interrenalin, thus relieving the cute symptoms and prolonging life by retarding the rate at vhich the intoxication progresses. This constitutes the basis pon which I have developed my treatment of Addison's disease nd thus far it has proven more hopeful and beneficial than other orms of treatment, heretofore employed, based upon the as-umption that the disease is due to lack of circulating epinephrin.

It must be remembered that Addison's disease is not identical vith the condition seen in completely adrenalectomized animals, xcept perhaps in rare cases where the disease develops as an cute condition in consequence of bilateral thrombosis or hemor-hage in the adrenal glands. Adrenalectomy results in total cute suppression of adrenal function, while Addison's disease sually represents a more chronic disturbance of adrenal func-on, the degenerative changes in the glands being the result of, r associated with, complicating disease (most commonly tuber-ulosis, sometimes malignancy). Nevertheless, the symptoms vhich indicate physiological disturbances referable to interfer-nce with adrenal function are quite similar in both cases. Ex-epting pigmentation, which does not occur in adrenalectomized nimals, the symptoms may be said to differ mainly in severity nd duration.

Clinically, Addison's disease is recognized by the presence of muscular and cardio-vascular asthenia (low blood-pressure), gastro-intestinal disturbances, pigmentation of the skin, and often the existence of nervous symptoms. I desire to add two symptoms, not heretofore described, which have proven of diagnostic value, in my experience. Upon moderate pressure in the costo-lumbar angle it is often possible to elicit local tenderness. Sometimes in addition to local tenderness the patient describes a dull ache or discomfort referred to the lower abdomen. This tenderness may be unilateral or bilateral. When unilateral I have suspected more recent or more extensive degeneration of the adrenal on that side and have verified this suspicion, a number of times, at autopsy. The other symptom that I have frequently observed in Addisonian patients was suggested to me by my studies on adrenal insufficiency, in animals. It is an *aversion to fatty food,* which occurs with the onset or in the presence of evidences of the intoxication. I had an opportunity of demonstrating the existence of this symptom this morning, in a case of Addison's disease under Dr. Lisser's care, at the hospital. This symptom is of considerable interest in view of our observation that profound congestion of the pancreas is almost always found, post-mortem, in adrenalectomized animals. I have also found the pancreas congested, at autopsy, in a number of cases of Addison's disease, especially if the patient presented a severe acute condition, which terminated fatally within a relatively short time.

The basal metabolic rate as a diagnostic index is in my opinion entirely without significance. Observations made in a number of our cases of Addison's disease have demonstrated that basal metabolism may be normal, plus or minus, in any or all stages of the disease. Obviously, therefore, as a criterion for demonstrating the presence of specific cortical activity in adrenal extracts, as employed recently by a Chicago worker, the basal metabolic rate can be of no greater value than it is as a diagnostic factor in Addison's disease. The presumption that the basal metabolic rate is subnormal in Addison's disease is probably based upon the erroneous concept that the disease is due to lack

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SUPRARENAL CORTICAL EXTRACTS IN SUPRARENAL INSUFFICIENCY (ADDISON'S DISEASE) *

of circulating epinephrin, the patient supposedly being deprived of the calorigenic influence of adrenalin.

Clinical blood studies, on patients with Addison's disease, reveal changes similar to those observed by us in adrenalectomized animals. There is frequently found an increase in the total non-protein nitrogen and urea nitrogen with a decided increase in the so-called undetermined fraction of the non-protein nitrogen. This is found more commonly and more strikingly during an acute exacerbation, or when the symptoms indicate increasing severity of the intoxication. Blood sugar is usually moderately reduced. Evidence of dehydration or concentration of the blood is usually present during an acute state in the disease.

Correlating our studies on adrenal insufficiency, in experimental animals, with my observations in Addison's disease, there is adequate support for the concept that I have so strongly emphasized, because it is not only the foundation upon which I have developed my treatment for Addison's disease but also constitutes the basis for further important studies on the physiology and pathology of the adrenal glands. From the available evidence the probability is great that the intoxication, which develops in consequence of lack of function of the interrenal gland tissue, gives rise to the characteristic symptoms by its influence upon the nervous system. This affords a satisfactory explanation for the co-existence of low blood-pressure, muscular asthenia, gastro-intestinal disturbances, and disturbances immediately referable to the nervous system. These include frequent weird and disturbing dreams, neurasthenia, psychasthenia and, in acute conditions, delirium, hallucinations, muscular spasm, convulsions, and coma. Perhaps, indeed, the serious metabolic disturbance which leads to the intoxication occurs in the nervous system.

It is possible that the pigmentation may indicate an attempt of the skin and mucous membranes to neutralize or destroy toxic material. This suggestion is based upon my observation that Addison's disease appears to be much more rapidly fatal in individuals in whom little or no pigmentation has occurred. Blondes

who develop adrenal insufficiency seem less capable of becoming pigmented, and I have been led to consider the prognosis in such individuals much more unfavorable. The absence of pigment usually renders the diagnosis of Addison's disease somewhat more difficult.

It has been sometimes said that Addison's disease can rarely be diagnosed until autopsy. I cannot agree with that opinion, for I have repeatedly demonstrated that, even in atypical cases, the diagnosis may be made with reasonable certainty. Indeed, I have quite often been able to predict, from clinical studies in a number of cases that have come under my observation, the nature of the pathology of the adrenals, which was later verified at autopsy.

Unfortunately, when diagnosis of Addison's disease can be made with reasonable certainty, the degenerative changes of the adrenal glands are already so extensive that complete cure of the syndrome could not be hoped for, with possibly rare exceptions, even if a specific cortical hormone were available in pure form. Our hope at present rests in the acquisition of more definite knowledge of the function of the interrenal gland tissue and the earlier recognition of disturbances of this function. Functional regeneration might then be more readily accomplished through the aid of substitution therapy. I may remark that my chief interest in clinical studies of Addison's disease is to facilitate these physiological investigations, with which I am engaged. It is unnecessary to claim positive or permanent cures from interrenalin in Addison's disease, to support the concept embodied in what I have thus far stated. Very satisfactory and hopeful progress is adequately indicated by a demonstration that life can be prolonged and symptoms ameliorated, in a good proportion of cases. Indeed, an alleged "cure" of a patient with Addison's disease, under any treatment, should at present lead to a strong suspicion that the patient would probably have been more correctly diagnosed as a "sun-tanned neurasthenic."

I have been unable, experimentally or clinically, to find any satisfactory evidence of beneficial influence of adrenalin in the treatment of adrenal insufficiency. Nor should any real benefit

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May 11, 1929, Vol. 92, pp. 1569-1571

SUPRARENAL CORTICAL EXTRACTS IN SUPRARENAL INSUFFICIENCY (ADDISON'S DISEASE) *

be expected from adrenalin if, as I am convinced, the symptoms of interference with adrenal function are not due to lack of circulating epinephrin. In my experience any sustained improvement of an Addisonian, under treatment with adrenalin, can be explained by the spontaneous remissions which may occur. I have observed, further, that administration of adrenalin, in typical cases of Addison's disease, very frequently gives rise to distressing symptoms. The so-called Muirhead treatment, vigorously defended by certain clinicians, which consists chiefly of administration of adrenalin, cannot, in my opinion, be said to have proven satisfactory in Addison's disease. Nor has it been helpful in the solution of physiological problems in adrenal insufficiency.

A number of cases of Addison's disease in which the diagnosis could not be doubted, that have come under my observation, had been treated by the Muirhead régime or administration of adrenalin without material benefit. Those in which such treatment was substituted by administration of interrenalin, with or without injections of physiological saline, showed unmistakable improvement. This will be evident from the tables which are to follow (7, 8, 9, 10). The groups of cases tabulated in these tables are much too small to permit deductions from averages; nevertheless, comparison of the columns headed "Duration" and "Progress" will afford ample support for my contention. Under "Duration" I have recorded the period from the first symptom, recognized in the history of the patient as a definite symptom of adrenal deficiency, until the present time in the case of those now undergoing treatment (Table 10) and until death in the others (Tables 7, 8, 9).

Of the cases now under treatment with interrenalin (some with saline injections in addition), represented in Table 10, and those who received this treatment but are no longer living (Table 9), eleven had been previously treated by the Muirhead régime, or by administration of adrenalin, without benefit. One case (Ald.) is included in Table 10, although I would hesitate to insist upon a positive diagnosis of Addison's disease. Nevertheless, his symptoms include asthenia (muscular and cardio-

vascular), gastro-intestinal disturbances, and pigmentation. I we may properly employ the terms "incipient" or "potential' Addison's disease he would undoubtedly belong to that class o cases. Other forms of treatment over a period of nearly tw years yielded no benefit, and the patient was gradually declining

TABLE 7

CASES REPORTED BY THOMAS ADDISON

Case	Age	Sex	Duration	Acute Exacerbation	Progress	Adrenals	Remarks
1....	32	M	2 yrs.	2	+—+—	Large; calcified	Pneumonia follo second exacerb n
2....	35	M	6 mos.	2*	Gradual decline	Tubercular degeneration	Gastric hemorrha petechiae
3....	26	M	6 mos.	1*	Gradual decline	Tubercular caseation and degeneration	Tuberculosis of l s; psoas abscess
4....	22	M	6 mos.	1*		Atrophy; fibrosis	Tuberculosis of l s
5†...	..	F	......	1*		L. suppurated; large; hard	Tuberculosis of l s (and g.i. ?)
6....	50+	M	1 yr.	1*	Gradual decline	Large; indurated; t. b. degeneration	Tubercular intest s?
7....	60	F	......			Cancer of both	Cancer; extensiv metastases
8....	53	F	......			Cancer of one adrenal	Cancer of stoma metastases
9....	58	M	......			Tuberculosis of one adrenal	Tuberculosis of k neys; etc.
10....	28	F	......			Thrombosis of one adrenal vein	Cancer of uterus; died in labor
11....	..	M	......			Cancer of one adrenal	Cancer; extensive metastases

* Exacerbation fatal
† Reported by Bright, in 1829.

The remarkable improvement of his condition under treatmen with interrenalin may be interpreted as an indication that marke functional insufficiency of adrenal cortex, if not anatomica deficiency, had existed.

Table 8 represents a group of cases not treated with interrenalin. Six of these patients were treated with adrenalin or a

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May 11, 1929, Vol. 92, pp. 1569-1571

SUPRARENAL CORTICAL EXTRACTS IN
SUPRARENAL INSUFFICIENCY
(ADDISON'S DISEASE) *

combination of adrenalin with desiccated whole adrenal. The influence of the treatment that I have employed in prolonging life and in the amelioration of symptoms, as well as the superiority of this treatment over any other hitherto tested in Addison's disease, is convincingly demonstrated by the data

TABLE 8

Cases not Treated with Interrenalin

ase	Age	Sex	Duration	Acute Exacerbation	Progress	Adrenals	Remarks
N ..	35	M	1½ yrs.	2*	+—+—	Atrophy; caseous degeneration	Tuberculosis of hip
...	41	F	1 yr.	2*	Gradual decline		Tuberculosis of hip
...	25	F	2 yrs.	8*	+—+—	Atrophic	Adrenalin and saline injections
r...	50	F	1¼ yrs.	1*	Gradual decline		Adrenalin injections
...	43	F	1 yr.	2*	Gradual decline		Adrenalin injections
...	36	F	6 mos.	2*	Rapid decline	Complete fibrosis	g.i. tract and pancreas++
a..	40	F	1½ yrs.	3*	Gradual decline	Tubercular hyaline	Tubercular (?)
l..	39	F	9 mos.	2	Gradual decline	Atrophy	Muirhead regime

* Exacerbation fatal.

recorded in the two columns previously mentioned (Duration; Progress). It may be remarked that the results compare very well with those obtained in my studies upon animals with experimental adrenal insufficiency.

In Table 7, I have tabulated the eleven cases reported by Thomas Addison in 1855. Careful analysis of his description of these cases has convinced me that not more than six of them can be properly included as genuine cases of adrenal insufficiency. In view of our present knowledge, there can be no doubt that none of the last five cases died from adrenal insufficiency. Four

TABLE 9

CASES TREATED WITH INTERRENALIN (GROUP A)

Case	Age	Sex	Duration	Acute Exacerbation	Treatment	Progress	Adrenals	Remarks
Mil.....	26	M	1½ yrs.	1	Interrenalin 5 months	Moderate improvement, then grad. decline	L. complete atrophy; R. calcified	Blood-pressure rose from 82 mm. to 106 mm.
Hum...	19	M	1 yr.	2	Interrenalin 5 months	+ improvement		Blonde; blood-pressure rose from 70 mm. to 98 mm.; influenza fatal
Kar....	48	M	2½ yrs.	2 or 3	Interrenalin 1 year	+++ improvement c; no exacerbations		Teeth extraction fatal
Smy...	26	F	3 yrs.	3*	Interrenalin 8 mos. discontinued 5 mos.	++ improvement during treatment	Hypoplasia; atrophy	Tuberculosis
Eat....	49	F	2 yrs.		Interrenalin 2 months	+ improvement during treatment	(Tumor)	Malignancy (?)
Lus....	50	M	2 yrs.	3*	Interrenalin (small dose)	+—+—		

* Exacerbation fatal.

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SUPRARENAL CORTICAL EXTRACTS IN SUPRARENAL INSUFFICIENCY (ADDISON'S DISEASE) *

TABLE 10

CASES AT PRESENT UNDER TREATMENT WITH INTERRENALIN* (GROUP B)

Case†	Age	Sex	Duration	Acute Exacerbation	Treatment	Progress	Blood Pressure Increase (Systolic)
Ada.......	32	M	1¾ yrs.	1	Interrenalin 9 months	++ improvement	From 82 to 108 mm.
Ald........	64	M	2 yrs.	..	Interrenalin 9 months‡	++++ improvement	From 92 to 110 mm.
Coc........	30	M	2½ yrs.	2	Interrenalin 9 months	+++ improvement	From 86 to 96 mm.
Gre........	28	F	3½ yrs.	2	Interrenalin 2 years	++ improvement	From 74 to 110 mm.
Mes........	39	M	3¼ yrs.	3	Interrenalin 1 year	++++ improvement	From 88 to 110 mm.
Opp.......	40	M	6 yrs.	2	Interrenalin 3 years‡	++++ improvement	From 88 to 108 mm.
Sne........	46	M	3 yrs.	2–3	Interrenalin 1½ years	++ improvement	From 90 to 100 mm.
Vac........	56	F	1 yr.	2	Interrenalin 6 months	++ improvement	No increase

* Pigmentation decidedly less in nearly all cases.
† Mes., Opp., and Ald., have returned to their regular occupations. The first, third, and last of these cases received saline injections.
‡ Treatment discontinued.

succumbed to cancer with extensive metastases, the other to dis seminated tuberculosis. In 4 cases, only one adrenal was ir volved. Existence of a relatively small mass of normal adren cortex, with intact circulation, would suffice to sustain life, unle it is assumed that total suppression of function is possible with out anatomical changes in the gland.

To what extent functional adrenal cortical insufficiency ma exist, without anatomical destruction of the glands, is not know However, there can be little doubt that various degrees of fun tional disturbance of these organs must be possible. An illustra tion has already been given in the case of Ald. (Table 10). have seen a number of patients, with moderate symptoms qui similar to those seen in more severe form in Addison's diseas sometimes including pigmentation, in whom it is impossible t attribute the condition to any definitely known cause. In suc cases, I believe it is quite justifiable to assume the existence c functional adrenal insufficiency or perhaps even to classify son of them as "potential" or "incipient" Addisonians. These cas usually respond in a very gratifying manner to administratic of interrenalin.

I have demonstrated that the treatment of Addison's diseas developed from my experimental and clinical studies in adren insufficiency, yields results which afford encouraging eviden that the concept which I have stressed, and upon which the trea ment is based, is fundamentally correct. In principle the trea ment consists of three phases: (1) treatment of the intoxic tion; (2) correction or prevention of metabolic disturbanc which lead to the intoxication; (3) treatment of underlying c associated conditions. The first phase is accomplished by intr venous administration of relatively large volumes of physi logical salt solutions; the second, by administration of pote adrenal extracts, representing the specific activity of the inte renal tissue (interrenalin). The third phase most common represents treatment for tuberculosis, but often this is not th only or most serious complication. This phase, of course, large determines the amount of benefit that may be expected fro treatment in any given case.

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SUPRARENAL CORTICAL EXTRACTS IN SUPRARENAL INSUFFICIENCY (ADDISON'S DISEASE) *

If Addison's disease were merely a manifestation of the mmediate effects of lack of a circulating hormone, the problem vould be much simpler. It may be supposed that if "inter-enalin" becomes available in pure form, treatment will be more iopeful, but it is obvious that until the physiology of the adrenals s sufficiently clear to enable early detection of disturbances in heir function the existence of such a hormone in pure form can ave no greater value than its use to facilitate physiological nvestigation.

In conclusion, I may sum up in a few words the present tatus of the physiology of the adrenal glands and its bearing pon Addison's disease. Secretion of epinephrin is not the in-ispensable function of the adrenals. The function which is ssential for life and health consists in the elaboration, storage, nd undoubtedly the secretion of a hormone (interrenalin) from he cortex or interrenal gland tissue. Interference with the latter unction is responsible for the syndrome known as Addison's isease. Treatment of this syndrome must, therefore, be based pon an attempt to correct the effects of cortical adrenal insuffi-iency. The important problem which at present confronts us is hat of obtaining more definite knowledge of the function of nterrenalin.

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It is generally assumed that Addison's disease and some other clinical conditions associated with low blood pressure are due to interference with epinephrine secretion from the suprarenal glands. This assumption is based on the fact that the medulla of these glands contains and secretes a blood pressure raising principle (epinephrine). However, the idea that these conditions result from a lack of this secretion is not supported by experimental evidence or by the results obtained in treating such cases by the administration of epinephrine. Our studies have led to the conclusion that we must consider Addison's disease (and other, less severe, forms of suprarenal insufficiency) as resulting from interference with the function of the *cortex* rather than the medulla of the suprarenal glands.

It has been established, experimentally, that epinephrine secretion from the suprarenals is not indispensable for life and good health, since it can be suppressed, in animals, without harmful effects.[1] But the cortex of the suprarenal (interrenal tissue) cannot be entirely removed or destroyed without a fatal outcome.[2] Further, it has been shown by us [3] that while

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1. Stewart, G. N., and Rogoff, J. M.: Quantitative Experiments on the Liberation of Epinephrine from the Adrenals After Section of Their Nerves, with Special Reference to the Question Whether Epinephrine is Indispensable for the Organism, J. Pharmacol. & Exper. Therap. **10:** 1 (July) 1917; Further Observations Showing that Epinephrine from the Adrenals is not Indispensable, Am. J. Physiol. **48:** 397 (May) 1919.

2. Rogoff, J. M., and Stewart, G. N.: Studies on Adrenal Insufficiency in Dogs: I. Control Animals Not Subjected to Any Treatment, Am. J. Physiol. **78:** 683 (Nov.) 1926.

3. Rogoff, J. M., and Stewart, G. N.: The Influence of Adrenal Extracts on the Survival Period of Adrenalectomized Dogs, Science **66:** 327 1927; Am. J. Physiol. **84:** 660 (April) 1928.

administration of epinephrine has little or no beneficial effect on suprarenal insufficiency, life can be prolonged and symptoms ameliorated, in animals deprived of the suprarenals, by administration of extracts prepared from the cortex of the glands. Our experimental work has yielded substantial confirmation of the view that the indispensability of the suprarenal glands depends on the function of the cortex, or interrenal gland tissue. Also, that this function is performed through elaboration of a hormone. Methods of extraction and purification of this product are still under investigation and will be published when sufficiently useful data are available. To distinguish such a hormone from the product of the medulla (epinephrine) and to indicate its origin in the *interrenal* tissue of the gland, we have adopted the name "interrenalin." In the course of our experimental researches on animals during the past fourteen years, we have had a number of opportunities to make some clinical observations and laboratory studies on human beings with Addison's disease. During the past eight years we have made some observations on the effects of various forms of treatment in this condition, more recently including administration of some of the cortical extracts that proved beneficial in suprarenalectomized dogs. The number of cases of Addison's disease available for treatment is not great enough to permit definite conclusions, but the encouraging results obtained, up to the present, are of sufficient interest to justify a preliminary report.

It must be borne in mind that, except for certain manifestations of suprarenal cortical deficiency, Addison's disease is not comparable with the condition that follows complete suprarenalectomy in animals. In the latter case there is total acute suppression of suprarenal function and absence of underlying or associated complications, while in Addison's disease, generally, there exists not only severe, yet more or less incomplete, anatomic and physiologic deficiency of the glands due to hypoplasia, degeneration and atrophy, but other conditions (most commonly tuberculosis) that are the cause of or are associated with the suprarenal condition. Obviously, this renders it more difficult to estimate the value of substitution therapy with glandular extracts, which, of course, can be expected to influence only the insufficient suprarenal function and must vary in its

beneficial effect with the amount of functioning cortex still present and also with the severity of underlying or associated conditions.

In animals, our suprarenal cortical extracts were administered intravenously, but in human beings only by mouth, in capsules coated to resist gastric digestion. More striking and more constant results may be expected both in animals and in man when more concentrated products are obtainable, and when appropriate dosage and the best method of administration can be determined. If earlier diagnosis of Addison's disease becomes possible, the result of treatment with the suprarenal cortical extracts might be even more hopeful.

We have no doubt that suprarenal cortical deficiency may exist in less severe form than that usually seen in typical Addison's disease. Occasionally, some or all of the characteristic symptoms of this disease may develop and apparently lead to spontaneous recovery. Another condition is sometimes seen, which may be considered one of incipient Addison's disease, in which there is a gradual development of the characteristic symptoms over a period of years, without a history of any of the acute phenomena that usually develop within a few months in typical cases. The existence, in a patient, of a systolic blood pressure below 100 mm. (especially if it is known to have been much higher during good health) associated with gastro-intestinal disturbances, increasing fatigue or muscular weakness, with or without noticeable pigmentation of the skin or buccal mucous membrane, should arouse suspicion of suprarenal cortical deficiency (or potential Addison's disease) if repeated careful examination of the patient fails to reveal some other reasonably definite cause for these symptoms. In such a case, if treatment with interrenalin proves beneficial it may be considered as additional evidence that impaired function of the suprarenal cortex was chiefly responsible for the condition. We have seen improvement in such cases under this treatment.

Although, as has been indicated, Addison's disease and the condition seen in suprarenalectomized animals are not to be considered identical, there are present in both cases certain symptoms which, though varying in severity, are qualitatively the same and are to be considered as a part of the manifestations of suprarenal insufficiency. One symptom commonly observed in

suprarenalectomized dogs and very frequently reported by patients with Addison's disease is an aversion to food rich in fat. Anorexia and gastric disturbances (including bilious vomiting) are common in the two conditions. Frequently, symptoms referable to the nervous system develop, especially in acute conditions. The low blood pressure associated with Addison's disease, which heretofore has been interpreted as an indication of interference with epinephrine secretion, is more probably a manifestation of the intoxication that develops as a result of deficient cortical function.

In cases in which the clinical diagnosis of Addison's disease seems fully justified, treatment with interrenalin has yielded results that may be considered promising. Even during acute exacerbations we have sometimes seen evidence of improvement in the symptoms referable to the suprarenal deficiency. The possibility of temporary spontaneous improvement or of psychologic effects simulating benefit due to the treatment cannot always be eliminated, but it is significant that when improvement is seen it generally occurs about two to four weeks after the beginning of treatment.[4]

Brief abstracts of the records from a few cases under treatment are given here. In case 1, the diagnosis may be doubtful but we do not hesitate to include it as an example of less severe suprarenal cortical deficiency, if not of definite Addison's disease. We give more details in case 2 because there is no doubt in the diagnosis and the improvement under treatment with interrenalin has been striking. As to the question of permanent recovery in any of the cases treated, nothing can be said until a much longer period has elapsed.

REPORT OF CASES

Case 1.—O., a man, aged 40, single, seen in October, 1927, had had meningitis in 1918, and during the next five or six years muscular fatigue had been brought on easily with moderate exertion, and nausea and vomiting had occurred frequently. The patient was of dark complexion, but for three or four years his skin had been getting decidedly darker. His weight at the time of examination was 116 pounds (53 Kg.). The symptoms consisted of anorexia, nausea, frequent bilious vomiting, aversion to milk and fatty food, insomnia and moderate but increasing muscular asthenia. The systolic blood

4. Since this article was written, we have observed improvement, within about two weeks, in two new cases (not included in this report).

pressure was 88. Physical and roentgen examinations of the chest were negative. Administration of interrenalin was begun at the end of December, 1927. Feb. 6, 1928, the systolic blood pressure was 98; the weight, 118 pounds (54 Kg.). The patient's appetite improved and he felt better. February 17, the blood pressure was 102 systolic and 74 diastolic; February 25, 106 systolic and 82 diastolic. March 31, the patient's weight was 121 pounds (55 Kg.) ; and the systolic blood pressure, 106. Treatment was discontinued, March 3. April 27, his aversion to fats was again present. May 19, the weight was 121 pounds; the blood pressure was 106 systolic and 82 diastolic; and his appetite was poor. He resumed treatment, and by June 16 his appetite had improved. June 30, his weight was 121 pounds; the blood pressure was 108 systolic and 82 diastolic, and the pulse rate was 68; he ate well (including fat meats and milk). From about the middle of July to October 20, treatment was intermitted, and nausea developed. November 30, the patient felt very well; he tolerated all kinds of food and drinks, having two or three glasses of milk daily; he slept well, and worked hard without becoming fatigued. Treatment was resumed, October 20. Jan. 19, 1929, the blood pressure was 108 systolic and 86 diastolic; the weight, 123 pounds (56 Kg.). The patient stated that his health was better than it had been for a number of years; appetite and digestion were excellent and he did not experience fatigue during work. The color of his skin was decidedly lighter than it had been before beginning treatment.

Case 2.—K., a man, aged 48, married, seen, March 25, 1928, stated that in the latter part of 1926 he began to lose weight. At that time his blood pressure ranged from 140 to 145 systolic and from 80 to 85 diastolic. Early in 1927, loss of weight continued, gastric disturbances developed, and muscular weakness compelled the patient to go to bed frequently. Pigmentation was noticed about March or April. The symptoms gradually increased in severity and frequency, and pigmentation became more pronounced and diffuse. In July, the systolic blood pressure was 80 mm. of mercury. Between August and November he was confined to bed most of the time, and during this period he had a number of acute exacerbations, including severe and frequent vomiting of bile and mucus (as often as from twelve to sixteen times daily), profound asthenia and exhaustion, and on one or two occasions severe nervous symptoms (hallucinations). Temporary improvement in the severity of the symptoms occurred with occasional remissions, but the general condition remained poor. In March, 1928, the patient was extremely emaciated, being unable to retain food or water most of the time. The systolic blood pressure was about 86 mm. He had profound muscular asthenia which confined him to bed constantly for about three months. Administration

of interrenalin was started, April 1. Statements of the family and attending physician are as follows:

June 4. "The past three weeks have shown quite an improvement in strength and appetite; vomiting has been greatly reduced although almost daily there is nausea; occasionally there is vomiting. Yesterday, he sat in a chair for twenty minutes, the first time in months."

July 28. "The patient sits up in a chair from two to three hours daily and feels better than he has for about two years; his appetite is good; nausea is less frequent and he is gaining weight; the color of the face seems lighter but the body is about the same."

September 1. "Blood pressure is 90 systolic and 50 diastolic."

October 1. "He has had more nausea and some vomiting within the past two weeks but he is again improving. His appetite is usually excellent, and he is eating all kinds of foods. He sits up from three to three and one-half hours daily without fatigue."

October 14. "During the past week he has had some nausea and occasional vomiting."

November 8. "The nausea has completely disappeared; the patient is feeling very well. He sat up eight and one-half hours the other day, all at one time, and without feeling unduly weary."

Feb. 11, 1929. "The patient had a bad cold about three weeks ago but is now fully recovered. He is feeling much stronger, walks about in the house with very little fatigue, eats well and is gaining in weight. The color of his skin appears to be getting lighter."

Case 3.—Mrs. G., aged 28, seen, Nov. 26, 1927, stated that during the latter part of 1926 she had been gradually losing weight and had frequently become fatigued. In the spring of 1927, pigmentation of the skin on the face and hands became noticeable and muscular fatigue occurred on slight exertion; gastric disturbances developed, nausea and vomiting occurring frequently. The symptoms continued and gradually increased in severity. Pigmentation of the skin became more diffuse and intense. At the time of examination the appetite was good, but frequently anorexia developed and at such times certain foods caused gastric upsets. Foods rich in fat were not tolerated. Physical examination of the abdomen revealed a firm, rounded, tender mass directly over the lowermost curvature of the spine; the thorax showed moderate increase of tactile and vocal fremitus over the right apex; otherwise physical, roentgen and blood examinations were negative. The systolic blood pressure was 96 mm. Some improvement occurred, spontaneously, but early in March, 1928, the patient complained that she was gradually getting weaker. Treatment with interrenalin was begun, March 16.

April 5, the weight was 116 pounds (53 Kg.); the blood pressure was 108 systolic and 90 diastolic.

April 17, nausea occurred less frequently; the patient felt stronger; the blood pressure was 112 systolic and 84 diastolic. She has gradually improved, with occasional recurrence of gastric symptoms and moderate fatigue on excessive exertion, her present weight being from 120 to 122 pounds (54 to 55 Kg.) and the systolic blood pressure about 112 mm. The mass in the lower part of the abdomen had entirely disappeared but recently again became palpable.

Case 4.—Miss S., aged 26, seen, Oct. 22, 1927, stated that during the latter part of 1925 she began to lose weight and tired readily. Early in 1926 asthenia became more pronounced, gastro-intestinal symptoms and pigmentation of the skin developed, and the systolic blood pressure was 83 mm., leading to the diagnosis of Addison's disease. In the spring of 1927 she had an acute exacerbation, including profound asthenia, severe gastro-intestinal disturbances, fever and nervous symptoms (delirium and hallucinations). Gradual recovery occurred with frequent remissions. Recently she had been having recurrence of gastro-intestinal symptoms and increasing fatigue. Administration of interrenalin was begun, Nov. 15, 1927. Improvement occurred about the middle of December. She left the city and treatment was discontinued during January and resumed, February 1. She continued in improved health and early in July she discontinued treatment, against instructions. An acute exacerbation, November 25, terminated in death, November 27. Postmortem examination revealed old tuberculous lesions in the apex of the right lung and more recent tuberculous processes in the upper part of the left lung. Careful search failed to reveal any tissue on either side that could be identified as suprarenal. Three or four very small nodular masses in the perirenal fat were discovered and the tissues over both kidneys were preserved for histologic examination of serial sections.

Case 5.—Mrs. E., aged 49, began treatment with interrenalin during an acute breakdown. Gastro-intestinal symptoms were severe and asthenia was very marked. Improvement was observed in about three weeks and continued during treatment for about a month thereafter. At this time administration of the cortical extract was discontinued by the attending physician, who desired to try other treatment.

Case 6.—S., a man, aged 46, married, had had definite symptoms of Addison's disease since the summer of 1927. Administration of interrenalin was begun, Sept. 7, 1928, during a period of decline. September 29, there was improvement in the gastro-intestinal condition, but the patient's appetite was poor. November 3, there was decided improvement in appetite, digestion and strength. He has since had occasional recurrence of nausea and some asthenia but has continued to improve, and there has been a gradual elevation of the blood pressure.

CASE 7.—H., a youth, aged 19, in May, 1928, following three days of influenza, began to notice persistent weakness and fatigue. He improved for a while but soon developed loss of appetite, shortness of breath on slight exertion and extreme prostration. During October and November he was removed to a hospital, where he was repeatedly examined (including roentgenographic and laboratory examinations) ; treatment with dilute hydrochloric acid, iron and arsenic, suprarenal gland and ephedrine was carried out, but he made no improvement. When seen in consultation (by J. M. R.) in the latter part of November, he had been confined to bed almost constantly for three weeks, because of extreme weakness and considerable nausea and vomiting. His blood pressure was 70 systolic and 48 diastolic. No diffuse bronzing of the skin was present, but a number of freckle-like dark spots on both sides of the neck, shoulders, arms and back and one small bluish patch on the mucosa of the left cheek were observed. Administration of interrenalin was begun. About two or three weeks later improvement occurred and continued, with one remission lasting three or four days. The blood pressure rose to 90 systolic and 50 diastolic. Jan. 14, 1929, the condition was decidedly better: the appetite was good; gastro-intestinal disturbances were absent; the patient did not fatigue readily and the systolic blood pressure was 98 mm. of mercury. Improvement has continued up to the present.

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It is generally assumed that Addison's disease and some other clinical conditions associated with low blood pressure are due to interference with epinephrine secretion from the suprarenal glands. This assumption is based on the fact that the medulla of these glands contains and secretes a blood pressure raising principle (epinephrine). However, the idea that these conditions result from a lack of this secretion is not supported by experimental evidence or by the results obtained in treating such cases by the administration of epinephrine. Our studies have led to the conclusion that we must consider Addison's disease (and other, less severe, forms of suprarenal insufficiency) as resulting from interference with the function of the *cortex* rather than the medulla of the suprarenal glands.

It has been established, experimentally, that epinephrine secretion from the suprarenals is not indispensable for life and good health, since it can be suppressed, in animals, without harmful effects.[1] But the cortex of the suprarenal (interrenal tissue) cannot be entirely removed or destroyed without a fatal outcome.[2] Further, it has been shown by us [3] that while

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* From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University School of Medicine.

1. Stewart, G. N., and Rogoff, J. M.: Quantitative Experiments on the Liberation of Epinephrine from the Adrenals After Section of Their Nerves, with Special Reference to the Question Whether Epinephrine is Indispensable for the Organism, J. Pharmacol. & Exper. Therap. **10:** 1 (July) 1917; Further Observations Showing that Epinephrine from the Adrenals is not Indispensable, Am. J. Physiol. **48:** 397 (May) 1919.

2. Rogoff, J. M., and Stewart, G. N.: Studies on Adrenal Insufficiency in Dogs: I. Control Animals Not Subjected to Any Treatment, Am. J. Physiol. **78:** 683 (Nov.) 1926.

3. Rogoff, J. M., and Stewart, G. N.: The Influence of Adrenal Extracts on the Survival Period of Adrenalectomized Dogs, Science **66:** 327 1927; Am. J. Physiol. **84:** 660 (April) 1928.

suprarenalectomized dogs and very frequently reported by patients with Addison's disease is an aversion to food rich in fat. Anorexia and gastric disturbances (including bilious vomiting) are common in the two conditions. Frequently, symptoms referable to the nervous system develop, especially in acute conditions. The low blood pressure associated with Addison's disease, which heretofore has been interpreted as an indication of interference with epinephrine secretion, is more probably a manifestation of the intoxication that develops as a result of deficient cortical function.

In cases in which the clinical diagnosis of Addison's disease seems fully justified, treatment with interrenalin has yielded results that may be considered promising. Even during acute exacerbations we have sometimes seen evidence of improvement in the symptoms referable to the suprarenal deficiency. The possibility of temporary spontaneous improvement or of psychologic effects simulating benefit due to the treatment cannot always be eliminated, but it is significant that when improvement is seen it generally occurs about two to four weeks after the beginning of treatment.[4]

Brief abstracts of the records from a few cases under treatment are given here. In case 1, the diagnosis may be doubtful but we do not hesitate to include it as an example of less severe suprarenal cortical deficiency, if not of definite Addison's disease. We give more details in case 2 because there is no doubt in the diagnosis and the improvement under treatment with interrenalin has been striking. As to the question of permanent recovery in any of the cases treated, nothing can be said until a much longer period has elapsed.

REPORT OF CASES

CASE 1.—O., a man, aged 40, single, seen in October, 1927, had had meningitis in 1918, and during the next five or six years muscular fatigue had been brought on easily with moderate exertion, and nausea and vomiting had occurred frequently. The patient was of dark complexion, but for three or four years his skin had been getting decidedly darker. His weight at the time of examination was 116 pounds (53 Kg.). The symptoms consisted of anorexia, nausea, frequent bilious vomiting, aversion to milk and fatty food, insomnia and moderate but increasing muscular asthenia. The systolic blood

4. Since this article was written, we have observed improvement, within about two weeks, in two new cases (not included in this report).

pressure was 88. Physical and roentgen examinations of the chest were negative. Administration of interrenalin was begun at the end of December, 1927. Feb. 6, 1928, the systolic blood pressure was 98; the weight, 118 pounds (54 Kg.). The patient's appetite improved and he felt better. February 17, the blood pressure was 102 systolic and 74 diastolic; February 25, 106 systolic and 82 diastolic. March 31, the patient's weight was 121 pounds (55 Kg.) ; and the systolic blood pressure, 106. Treatment was discontinued, March 3. April 27, his aversion to fats was again present. May 19, the weight was 121 pounds; the blood pressure was 106 systolic and 82 diastolic; and his appetite was poor. He resumed treatment, and by June 16 his appetite had improved. June 30, his weight was 121 pounds; the blood pressure was 108 systolic and 82 diastolic, and the pulse rate was 68; he ate well (including fat meats and milk). From about the middle of July to October 20, treatment was intermitted, and nausea developed. November 30, the patient felt very well; he tolerated all kinds of food and drinks, having two or three glasses of milk daily; he slept well, and worked hard without becoming fatigued. Treatment was resumed, October 20. Jan. 19, 1929, the blood pressure was 108 systolic and 86 diastolic; the weight, 123 pounds (56 Kg.). The patient stated that his health was better than it had been for a number of years; appetite and digestion were excellent and he did not experience fatigue during work. The color of his skin was decidedly lighter than it had been before beginning treatment.

Case 2.—K., a man, aged 48, married, seen, March 25, 1928, stated that in the latter part of 1926 he began to lose weight. At that time his blood pressure ranged from 140 to 145 systolic and from 80 to 85 diastolic. Early in 1927, loss of weight continued, gastric disturbances developed, and muscular weakness compelled the patient to go to bed frequently. Pigmentation was noticed about March or April. The symptoms gradually increased in severity and frequency, and pigmentation became more pronounced and diffuse. In July, the systolic blood pressure was 80 mm. of mercury. Between August and November he was confined to bed most of the time, and during this period he had a number of acute exacerbations, including severe and frequent vomiting of bile and mucus (as often as from twelve to sixteen times daily), profound asthenia and exhaustion, and on one or two occasions severe nervous symptoms (hallucinations). Temporary improvement in the severity of the symptoms occurred with occasional remissions, but the general condition remained poor. In March, 1928, the patient was extremely emaciated, being unable to retain food or water most of the time. The systolic blood pressure was about 86 mm. He had profound muscular asthenia which confined him to bed constantly for about three months. Administration

CASE 7.—H., a youth, aged 19, in May, 1928, following th days of influenza, began to notice persistent weakness a fatigue. He improved for a while but soon developed loss appetite, shortness of breath on slight exertion and extre prostration. During October and November he was remov to a hospital, where he was repeatedly examined (includi roentgenographic and laboratory examinations); treatment w dilute hydrochloric acid, iron and arsenic, suprarenal gland a ephedrine was carried out, but he made no improvement. Wh seen in consultation (by J. M. R.) in the latter part November, he had been confined to bed almost constantly f three weeks, because of extreme weakness and consideral nausea and vomiting. His blood pressure was 70 systolic a 48 diastolic. No diffuse bronzing of the skin was present, b a number of freckle-like dark spots on both sides of the nec shoulders, arms and back and one small bluish patch on t mucosa of the left cheek were observed. Administration interrenalin was begun. About two or three weeks lat improvement occurred and continued, with one remission lastir three or four days. The blood pressure rose to 90 systolic ar 50 diastolic. Jan. 14, 1929, the condition was decidedly bette the appetite was good; gastro-intestinal disturbances we absent; the patient did not fatigue readily and the systol blood pressure was 98 mm. of mercury. Improvement ha continued up to the present.

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INFLUENCE OF ADRENAL EXTRACTS ON THE SURVIVAL PERIOD OF ADRENALECTOMIZED DOGS[1]

IL it has been shown that any adrenal extracts ver possess the power of definitely increasing riod of survival after removal of the adrenals, sumption that the cortex produces a "hormone" n might appropriately be termed interrenalin) lack a foundation, and attempts to isolate and such a body can hardly be made with confi- We have used extracts, made from dog's adrenals with 0.9 per cent. salt solution and ine. The clear extracts were injected intraven- on alternate days. The injections produced no s ill effects.

only criterion at present at our disposal to ine the efficacy of an extract is its effect on the al period. This is a very severe test because fact that all the important derangements which ally lead to death must be neutralized by the nce. If changes not of themselves causing could be associated with the loss of the adrenals, ht be easier to obtain evidence of the existence acts of a body or bodies capable of preventing

obvious that in drawing conclusions as to the of any method of treatment upon the period of l, it is essential to have a sufficiently large r of "control" animals. We have accumulated han a hundred control dogs, so that it may l with confidence that we know the limits of vival period in dogs doubly adrenalectomized and not subjected to any treatment. Of course, this series of control animals is necessary and available for many other researches. The majority of the animals lived about a week to 10 days, a good many less than a week. About 8 per cent. survived a fortnight or somewhat longer. The longest survival period was $16\frac{1}{4}$ days (one dog).

Among about 30 dogs treated with extracts, one lived into the 18th day, one into the 20th day, one into the 22nd day, one into the 23rd day, one into the 28th day, and one survived 78 days after removal of the second adrenal. Nothing like those results were seen among the much larger number of control dogs. It is impossible to draw any other conclusion than that the extracts in some way prolonged the life of the animals in the absence of the adrenals. The rest of the treated animals compared favorably with the controls as regards duration of survival. As the extracts injected into the different animals were often obtained from different adrenals, it is easily understood that their potency would vary.

There is no reason to suppose that the epinephrin, present in larger or smaller amount in the extracts, could have had any appreciable influence in prolonging life. No effect of this kind was observed when epinephrin equal to the maximum amount which could have been contained in the dose, given on the assumption that none of it had been destroyed, was injected. Much of the epinephrin was destroyed in making the extract.

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STUDIES ON ADRENAL INSUFFICIENCY

THE INFLUENCE OF INTRAVENOUS INJECTIONS OF RINGER'S SOLUTION UPON THE SURVIVAL PERIOD IN ADRENALECTOMISED DOGS

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hese experiments were undertaken with the object of gaining informa- on the pathology of the condition which develops after removal of the enals and which culminates in a more or less characteristic train of ptoms described in detail in a previous paper (Rogoff and Stewart, 5). Another purpose was to lengthen, if possible, the period of survival drenalectomised animals so that they could be studied, in various ways, a longer time. All the experiments were performed on dogs. When egan the work, all statements in the literature greatly underestimated survival period. Our control animals, i.e., those in which no treatment adopted, constitute a series of more than 100 dogs that has been uired over the period of years during which we have been engaged in ies on adrenal insufficiency.

is absolutely necessary to have such a series, as the statements in the ature as to the survival period are vitiated by inadequate surgical nique. This is true not only for dogs but also for other animals. writer who compares his results in animals "treated" in any way, with nstance, the notoriously inaccurate results of Strehl and Weiss in , cats, etc., is certain to be in error. This is illustrated by quite recent :.

paper I of this series (Rogoff and Stewart, 1926) we have given on the length of survival and the period of good health after adre- ctomy in 74 dogs that were not subjected to any treatment. In her article (paper V of this series), will be published additional data 6 control animals. A very small number of the control dogs lived t a fortnight after removal of the second adrenal. The majority ived about a week. With this large number of controls for comparison not difficult to conclude with confidence whether the survival period

[1] preliminary account of this investigation was published in Proc. Soc. Exper. and Med., 1925, xxii, 394.

has been influenced by any given treatment or not. Untreated dogs n
survive the onset of the characteristic terminal symptoms more tha
very few days. These symptoms suggest a severe and increasing intox
tion. The nature of the toxic substance or substances is unknown,
it was thought that important suggestions might be derived if injection
simple salt solution could be shown to exert a favorable action upon
symptoms and survival period. These experiments were begun in 1
and continued through 1925 and the early part of 1926.

TABLE 1

Adrenalectomised dogs treated by intravenous injections of Ringer's solution

RECORD NUMBER	WEIGHT		INTERVAL BETWEEN OPERATIONS	SURVIVED		BEGAN TO REFUSE FOOD	ADRENAL WEIGHTS		ALIMENTARY CANAL		PAN CON TI
	First operation	Second operation					Right	Left	Blood	Congestion	
	kgm.	*kgm.*	*days*	*days*	*hours*	*days*	*gram*	*gram*			
86–2			12	19	12	8			+		-
96–6	9.5	10.2	6	19	8	10	0.60	0.65	+−	+−	
97–8	11.7	12.9	27	33	5	16	0.62	0.56	+++	+++	+-
98–0	11.5	11.0	12	14	20	13½	0.65	0.54	++	++	+
100–2	11.6	13.3	13	32	17	26	0.78	0.90	++	++	+
100–5	8.1	8.3	12	15	5	11	0.45	0.45	+	+	++
102–6	11.5	13.3	39	17	6	14½	0.75	0.85	+++	+++	+-
113–1	9.2	8.5	31	6	16	5	0.40	0.37	++	++	++
113–3	10.2	9.3	31	5	12	5	0.57	0.66	0	+	+-
113–4	9.4	9.5	53	14	4	4½	0.58	0.48	+−	0	+
114–0	12.4	12.6	14	13	15	7	0.70	0.60	++	+	+
117–0	11.1	10.3	33	53	16	45	0.70	0.60	+−	0	++
117–2	7.7	7.5	33	38	2	19	0.65	0.72	+	+	++
118–3	11.2	10.8	18	20		19	0.55	0.60	++	+++	++
118–4	6.8	7.5	30	20	3	15	0.65	0.80	++	++	-
119–0	10.7	10.8	23	3		3	0.73	0.85	+	++	++
119–1	9.1	8.8	23	6	12	6	0.72	0.86	0	0	+-

All dogs are males except 100–2, 102–6, 113–4, 118–3 and 118–4. Right adrena
cised first in all the dogs except in 113–1, 113–3, 113–4 and 114–0.

In the large majority of our experiments the injections were given
daily, by intravenous route. Ringer's solution, having the follo
composition, was used—sodium chloride 9 grams, potassium chloride
gram, calcium chloride 0.24 gram and sodium bicarbonate 0.1 gram
1000 cc. of water. To this was added, in most cases, 2 to 3 gram
dextrose per liter, in a number of cases 5 to 10 grams per liter. The
administered was usually about 100 cc. per kilogram of body we
injected during a half to three-quarters of an hour. The dose was
posely large in order to produce a decided effect. Free mictura

occurs immediately or soon after the injection. Evidence of increased intestinal peristalsis is present, and commonly defecation occurs. Occasionally there is vomiting.

Table 1 shows clearly that the period of survival, after the second adrenalectomy, is often much increased beyond the maximum period for control untreated dogs. Of the 17 animals included in the table more than half lived into the 18th day or longer. Three of these lived between 30 and 40 days and one survived for nearly 54 days. No such results were obtained in any of the large number of controls, very few reaching 14 or 15 days and the great majority dying in 7 to 10 days or less. This is conclusive evidence that life can be prolonged by these injections. As to the proportion of successful cases and the absolute prolongation of life, it is not to be supposed that we obtained optimum results. The dose was varied but little, except that the larger animals received a larger volume than the smaller. Variation in the amount or frequency of the injections might have given better results. One injection was nearly always given in the twenty-four hours. But when an animal was failing, several injections were sometimes given, with benefit. The rule was to start the treatment about 24 hours after removal of the second adrenal. We acquired the impression that it was advantageous to begin the treatment early. Still it may well be that a better result might have been obtained had the first injection been postponed longer than 24 hours after an operation so serious as adrenalectomy. It was not determined whether variations in the amount of dextrose affected the result. The work was too laborious and time-consuming to permit us to attempt more than the definite proof that the treatment exerted a marked influence on the survival period and the period of good health.

The immediate beneficial effects of the treatment often observed in animals already showing severe symptoms, including coma, are well illustrated in some of the protocols, e.g., that of animal 102–6, the protocol of which has already been published in paper III (Rogoff and Stewart, 1927). At that time our studies on the influence of pregnancy (and rut) on the survival period and the period of good health after adrenalectomy had not been made. It is scarcely possible to determine the relative importance of this factor and of the salt solution, and therefore animals 102–6 and 100–2 have been included both in table 1 of paper III and in table 1 of this paper. The protocol of 102–6 indicates that in this animal the injections were not only important but actually restored the animal when comatose and permitted its survival in good condition for a relatively long period, death occurring after $17\frac{1}{4}$ days. Animal 100–2 survived 32 days $16\frac{1}{2}$ hours. A description of a few typical experiments with condensed protocols will now be given.

Dog. Male. Record number 86-2. October 19, 1924. Right adrenal excise November 10, left adrenal excised at 2:00 p.m. On November 11, in good conditio daily intravenous injections of Ringer were begun (1000 to 1400 cc., approximate 100 to 150 cc. per kgm. body weight with 2 grams dextrose per liter). November 1 reflexes exaggerated; tremor of leg muscles. Emesis after injection, later copio micturition. November 14, tremor of head and legs, relieved by the injection, whi was followed by copious micturition and defecation (semi-liquid stools). Novemb 14 to 18, condition very good; eating well and behaving normally. Temperatu varied little from 38°C. No injection given on November 18 and 19. On Novemb 19 total anorexia; has voided very little urine; lethargic and somewhat astheni Temperature 37.8°C. Restored to excellent condition by a Ringer-dextrose inje tion, which, as usual, was followed by diuresis and defecation. Weight 7.5 kgm lost 1 to 1½ kgm. since last operation, but in good health. Injections continued f remainder of survival period. November 20 and 21, condition good. November 2 some asthenia, muscular twitching; moderate anorexia. November 23, hallucin tions. November 24, 9:00 a.m. Legs spastic, reflexes exaggerated. Hallucinatio and short tonic spasm, followed by marked asthenia. At 11:30 a.m., injection fo lowed by improvement. At 9:30 p.m. another injection with marked improvemen November 27, total anorexia for the past 3 days; asthenia recurring and relieved b injections. Today he regained moderate appetite. Condition unchanged ti November 29, when asthenia became much more pronounced than heretofore. A 5:00 p.m., an injection was followed by temporary improvement. Died during nigh (November 29 to 30).

Dog. Male. Record number 96-6. November 18, 1924, right adrenal excise November 24, left adrenal excised. November 25. Condition excellent; appetit voracious. Daily Ringer injections begun (about 100 cc. per kgm. body weight wit 2 grams dextrose per liter). December 3, health excellent up to date. December refused food; moderate asthenia. On December 5 and 6, asthenia was relieved b injections, but total anorexia persisted. December 7, has regained some appetite fo certain foods (salmon, rabbit); asthenia no longer present. December 8 and 9, tot anorexia, but no asthenia. December 10, total anorexia; marked asthenia; letharg cough; emesis (bile-stained material) and defecation (some tarry feces) about a hour after the Ringer injection. December 11, no change; some improvement fo lowing injection. December 12 and 13, very lethargic and asthenic. Restored t fairly good condition after injections. December 13, 10:00 p.m., comatose; die during the night.

Animal 86–2, which survived 19½ days was still in excellent conditio on the eighth day after removal of the second adrenal, having receive daily injections of Ringer-dextrose solution. On this day it was decide to omit the treatment. The following day the animal was worse. H became lethargic, moderately asthenic and developed total anorexia But he was again restored to good condition by an injection of Ringer dextrose solution and the daily injections, thereafter, continued to benefi the animal up till shortly before death.

This dog and animal 96–6, which survived 19⅓ days after removal o the second adrenal, afford good illustrations of the fact brought ou clearly in table 1, that the interval between the first refusal of food an

death of the animal may be much greater in the animals treated by avenous administration of salt solution than in control, untreated nals. Thus animal 86–2 refused food 8 days and animal 96–6 refused 0 days after removal of the second adrenal, although life was still onged for 11 and 9 days respectively. In a number of the animals rexia and other symptoms were recovered from, repeatedly, under the ience of the injections.

ı animal 97–8, which survived more than 33 days, there was total rexia for the first 5 days after the second adrenalectomy. On the d day he developed coma with convulsions. The immediate beneficial ience of Ringer injection (which was given three times on this day) only temporary, but the ultimate result was very striking. The wing morning we found the animal walking about and normal in earance and behavior. In control, untreated adrenalectomised nals the occurrence of coma with convulsions presages death within atively short time. Appetite appeared on the fifth day and continued l till the sixteenth day. Then food was refused, and although some tite was regained it remained capricious and not nearly equal to nor- For the last 5 or 6 days of life there was total anorexia.

og. Male. Record number 97-8. Right adrenal excised December 18, 1924. adrenal excised January 14, 1925, at 2:30 p.m. Weight 12.85 kgm. January 15, to 10:30 a.m., Ringer 750 cc., with 2.5 grams dextrose. Urinated copiously; is about 15 minutes after injection, and again later. Total anorexia. January otal anorexia but seems strong; 10:30 a.m., temperature 39.3°C., pulse 152 (regu- Ringer 750 cc. with 2 grams dextrose. Urinated and defecated.

nuary 17, seems weaker; small stitch infection. 2:15 p.m., 750 cc. Ringer with ms dextrose. Urinated small amount. 5:30 p.m., tetanic convulsion and deep ; Ringer 1250 cc. with 3 grams dextrose. Urinated on table and afterwards ed much improved, but lethargic. 9:00 p.m., tetanic convulsion and deep coma; er 1000 cc. with 15 grams dextrose. Urinated, but became only slightly more ious during injection. At 9:30 p.m., deep coma.

nuary 18. In the morning he was standing up and wagging his tail. 3:00 p.m., tion much improved; Ringer 1500 cc. with 5 grams dextrose. Urinated. Le- ic after injection. January 19, much improved. Took food. Growled at other 5:00 p.m., 1400 cc. Ringer with 3 grams dextrose. Copious urination.

ll January 28, he remained in good condition, eating well. Received daily, c. Ringer with 2 to 3 grams dextrose. As on previous occasions he was lethargic n hour or two after the injection. Temperature 38.8°C. to 39.1°C. Stitch d healed.

nuary 29. Appetite less than usual; less alert. January 30. Very weak and olent; ate nothing. Not much urine voided. Temperature 38.4°C., pulse 88). Weak in hind quarters. January 31, somewhat better. February 1, much oved; urinated well; fair appetite. Runs about the room as usual. February , fairly active; some appetite but not as good as a week ago. Weight 10.85 kgm. iary 5, eating better, and February 6 worse. Same on February 7; weak in hind On February 8, ate some salmon. February 9, ate rabbit; ran about as usual. on February 10. No marked asthenia. February 11, took a little food but ed it up.

February 12, complete anorexia; gradually developing asthenia. When exci (by presence of a stranger), the hair on back and tail became erect and he bar violently and ran around. Apart from this he was more listless than usual toc Same on February 13. Took a run in the hall. Refused food. Temperature 38.2' pulse 100, respiration 27. February 14 and 15, not much change; some vomit Ringer (1000 cc. with 3 to 6 grams, once 8 grams, dextrose) was given daily f: January 28 onwards. Died at 7:00 p.m. on February 16, 1925.

Similar observations were made in other animals, including 117–0 : 117–2.

Dog. Male. Record number 117-0. January 6, 1926, weight 11.1 kgm., exc. right adrenal. February 8, 1926, weight 10.3 kgm., excised left adrenal at 2:00 ı

February 9, excellent condition. At 3:30 p.m., 850 cc. Ringer with 6 gr. dextrose. Urinated copiously. February 10 to March 8, received 1000 cc. Ri with 6 to 8 grams dextrose daily. Remained in excellent health. Pugnaciou usual. Large appetite. Always urinated largely after injection. Occasiona emesis of food taken soon after injection. Ate it up again.

March 9, slightly less active. Ringer 1000 cc. with 8 grams dextrose. March weight 9.7 kgm. Emesis in morning (very green acid liquid). 12:00 m., ate v 5:00 p.m., 1000 cc. Ringer with 8 grams dextrose. Some stretching, but condi good. March 11, unchanged. March 12, still eating, no asthenia, but beginı apathy. Injection continued daily. March 13 to 21, eating well; health gc weight 9.5 kgm. Ringer 1000 cc. with 10 grams dextrose daily. Emesis once or tv (some bile). March 21 and 22, appetite good. Tried to copulate several times.

March 23. Slow in eating meal (meat) but in time finished it. Ringer 1000 with 10 grams dextrose. Good micturition; some emesis. Heart very slow anc regular before injection, which improved it. March 24. Apathy more marl Ate meat. Green stool. Straddles slightly in walking. Some diarrhea after jection. No noticeable asthenia. March 25, fair condition but refused food. D rhea. Apathetic. Not asthenic. Somewhat improved after Ringer (1000 cc. v 10 grams dextrose). March 26, weight 9.2 kgm. Heart about 36 a minute, irregu Greenish stools with very offensive odor. Ate only a little meat. March after injection the heart rate went up from 36 to 140 to 150 a minute, but he did micturate for 2 hours after injection. Took no food. Emesis (bile-containing, ; liquid). March 28. Quite weak. Heart irregular and slow, not improved by jection, although he walked better. March 29 to 31. Ringer continued (1000 with 12 grams dextrose), and considerable improvement occurred. He ate some n and rabbit several times and with good appetite. Walked much better. Apri refused meat (rabbit). Urinated copiously during injection (1000 cc. Ringer v 15 grams dextrose) and afterwards. April 2. Poor condition. Some emesis. W on legs. Improved by injection. Urinated well. Walked. April 3, died, proba about 6:00 a.m.

Autopsy. Pancreas much congested. Stomach distended with bile-contair fluid. Practically no congestion. Same for duodenum, jejunum, ileum and col Rectum contained a small amount of blood-stained feces. Bladder distended v urine.

Dog. Male. Record number 117-2. January 6, 1926, right adrenal excis February 8, 1926, excised left adrenal at 2:30 p.m. From February 9 onwards, da injections were given (Ringer 650 cc. to 800 cc., with 6 to 8 grams dextrose). Hea

d till February 19, with the exception of emesis (bile-containing material) on ruary 17, on which day the animal was somewhat lethargic and did not have much etite. No asthenia. From February 20 to March 4, condition was very good t of the time. At intervals there was total or partial loss of appetite, sometimes sis (bilious) and he slept more than usual. But always his condition returned to nal. On March 5 to 7, apathetic with loss of appetite, increasing to total ano-a. No asthenia. March 8, appetite fair, otherwise unchanged. March 8 to 16, e change. At times apathetic and lethargic; some loss of appetite with occasional sis. No asthenia. March 17, slight asthenia (in hind quarters), improved after ction. No appetite. March 18, asthenia increased. Total anorexia. Coma 1 a convulsion; improved after injection. March 19, died early in the morning.

'he continued survival, in good condition, of the animals in spite of rexia, even when total, seems to us to be a suggestive point in the action he Ringer-dextrose solution. Anorexia, it seems, need not be accom-ied by the other serious symptoms which in the control animals presage th. That it can appear long before the fatal termination supports the v that it is only one of a number of symptoms indicating the profound angement ultimately produced by adrenal insufficiency. The manner vhich anorexia is modified by the injections explains the, at first sight, zling variations in the column in table 1, headed "Began to refuse food." y the first definite refusal of food is noted there.

Ve have already expressed the opinion that animals dying from adrenal ifficiency never die of lack of nutriment. This is true even when the od of anorexia is prolonged to several or many days under the in-nce of injected salt solutions. The treatment, in such cases, dissociates anorexia from the group of fatal symptoms. The loss of appetite is sent with the accompanying changes, whatever they may be, but that s not suffice to cause death. There is no reason to believe that the ll part of the caloric requirement covered by the injected dextrose le any essential difference in the survival period. No beneficial effects e observed in a small number of animals that were treated by sub-aneous administration of dextrose (5 to 10 grams dissolved in a small ime of salt solution, administered daily).

'he modification of the symptomatology, for example the postponement anorexia or recovery of appetite after total anorexia has appeared, is as important a proof of the beneficial influence of the injections as marked prolongation of life. The pathological appearances in the nentary canal, although apparently not in the pancreas, are also miti-ed by the injections as can be seen by comparing table 1 of this paper 1 tables 1 and 2 of paper I.

1 our preliminary paper (Stewart and Rogoff, 1925) something has 1 said of possible ways in which the injections may be beneficial. We ussed especially the possibility that a poison or poisons accumulating he absence of the adrenals may be washed out of the tissues (or perhaps

neutralised) by the thorough irrigation caused by the large injections Ringer's solution. If the injections are delayed till the concentration the blood described in a previous paper (Stewart, 1926) has occurred, t mere dilution may be expected to aid the circulation and thus produce beneficial effect.

If it were practicable to wash out all the poison it is conceivable th animals would survive indefinitely. In our experiments it may be assum to accumulate little by little, anchoring itself perhaps particularly in t nervous system, and also in the mucosa of the stomach and intestin which may be crippled at last and cease to eliminate it. That the muco is shielded in some way through the injections, seems to follow from t way in which anorexia and gastro-intestinal symptoms generally a staved off. An additional support to this view may be derived from t fact that the hemorrhagic condition observed *post mortem* in the alimenta canal seems to be less common and less severe than in the controls. Th can be seen by comparing table 1 of this paper with tables 1 and 2 of pap I. The difference can hardly be explained as due to the washing out blood from the vessels of the mucosa by the injected solution. For t congestion of the pancreas is usually as marked in animals which ha received injections as in the untreated controls. In a few experiments administered large quantities of water by stomach tube and in othe repeated gastric and colonic lavage was performed. No beneficial effec resulted from treatment of adrenalectomised dogs by these methods.

What part, if any, the kidneys take in the elimination of the hypothetic poison cannot be stated. No definite lesion has hitherto been discovered this organ in dogs. That the absence or delay of micturition after a injection is of sinister significance is true, but this may be due to circulato deficiencies and not to any pathological change in the kidneys. It mu be borne in mind that the first injection was made approximately 24 hou after the serious operation of adrenalectomy. Yet it is surprising ho easily the large injections are handled by the circulation. The outpu of the heart is, of course, much increased, but we never saw an instan in which breakdown occurred, due to dilatation of the organ, during immediately following an injection.

In the great majority of the experiments, we saw either tempora amelioration of the condition of the animal or survival for a period d cidedly beyond anything observed in untreated controls. But occasional animals were encountered which died with acute symptoms in a few hou after a single injection, given according to the usual routine, about 24 hou after removal of the second adrenal. In some of these it was thought th the Ringer's solution, kept for some time, might have been at fault. Bu fresh samples made with water from different sources, behaved in the sam way. Different samples of dextrose were also investigated, but witho

y definite result. It is possible that a more extensive investigation in we could afford time for would reveal the cause of the apparent uping of some of these exceptional results. That it was not due to a erence in the injection liquid is indicated by the fact that some animals ated with the same solution showed the usual behavior, increased vival period with mitigation of symptoms, while at the same time others d in a few hours. The fact that some of these animals did not micturate ing or after the injection indicates that the greatly increased volume iquid was not being properly handled from the beginning, although the al symptoms might not appear for some time. Whether dilatation the heart was responsible was not determined. A breakdown of the al mechanism is a possibility. It is unknown whether excretion of iid into the gastro-intestinal tract took place, although it is to be supled that it did, since defecation (frequently diarrhea) after the injection ally occurred as in the other dogs. In some also there was emesis ious). Of about half a dozen animals, constituting the exceptional up, three were somewhat out of sorts at the time of, or immediately er, the second operation (cough; in one instance with nasal discharge). ree were apparently in normal health. A protocol from animal 115–3 illustrate the course of events in this group.

og. *Male. Record number 115-3.* December 7, 1925. Weight 8.3 kgm.; right enal excised. December 16. Weight 8.1 kgm. At 1:45 p.m., left adrenal exd. December 17, condition excellent; pugnacious; good appetite. At 3:00 p.m., ger 750 cc. with 7.5 grams dextrose. Urinated and defecated (soft, yellow stool). :30 p.m., urinated; emesis. At 4:00 p.m., lying down, apathetic. At 5:30 p.m., sis had occurred (frothy mucus). Unsteady gait. Semi-stupor. Hallucinas; ran about in cage, aimlessly. At 7:30 p.m., increasing coma and convulsions. d at 7:40 p.m. *Autopsy* at once. Thorax normal. Heart contracted. Some id in abdominal cavity. Practically no congestion in gastro-intestinal mucosa pt in rectum. No blood. Pancreas much congested. Kidneys congested.

ne dog (114–2), which died $3\frac{2}{3}$ hours after a single Ringer injection a considerable amount of blood in the peritoneal cavity, probably n bleeding at the site of the second adrenal operation. Although it is ly that the loss of blood was not an important factor in causing death, animal was not included in the group. It is possible that with the ease in blood pressure due to the injection, a ligature may have slipped. the time of injection the dog seemed in very good condition. It took d (bread and milk) 10 minutes after the injection, but there was emesis r (bilious). The animal received somewhat less than the usual amount olution (60 cc. per kgm. with 8 grams dextrose). There was practically congestion of viscera except the pancreas, which was markedly coned. In two of the dogs, apparently in good health at the time of the ction, edema of the lungs was found *post mortem*. This and the in-

stance of liquid in the abdominal cavity, already mentioned, we consi evidence that the injection was not handled normally by the tissues.

Some control animals were used to test the question whether da injections of such quantities of Ringer-dextrose solution as were employ in the adrenalectomised animals had any effect. No effect was observed normal animals, except, of course, micturition. In one dog, afterwa adrenalectomised (98–0, table 1, paper I), the animal received, daily, injection of from 1200 cc. to 1750 cc. Ringer (100 to 150 cc. per kilo body weight) with 2 to 3 grams dextrose, for 10 days, without har The animal was then adrenalectomised in two stages in the usual way a used in the control series of adrenalectomised dogs. Another dog (115 table 1, paper I) after removal of the right adrenal, received daily injecti of Ringer (750 to 850 cc. (100 cc. per kgm.) with 5 to 10 grams dextro daily for 8 days, beginning with the day after the adrenalectomy. Th was occasional emesis and diarrhea; the appetite varied, although to anorexia was not seen. As some respiratory symptoms were prese after the injections, these were discontinued. The respiratory sympto disappeared after about a week except some nasal discharge, and the seco adrenal was removed. The experiment furnished no evidence that t injections, begun at so early a period after removal of one adrenal, had a harmful effects, unless they might have contributed something to t respiratory trouble.

While we are unable at present to explain a small group of exceptio results (3 animals apparently in good health when the injection was giv we do not consider that they constitute a contraindication for the the peutic use of the procedure in certain emergencies, in patients. The do in proportion to body weight, would be much smaller than was given to t dogs. It would probably be better to repeat the injection at shorter tervals than to give a very large quantity at one time. In some respe the conditions would seem to be more favorable in human cases than in t animals. No operation would have preceded the injection, and so cortical tissue would generally be present and functioning. Let it repeated, injection of salt solutions could be contemplated only in emerg cies, since they could not "substitute" for a missing hormone. It may however, that when cortical extracts were being administered an injecti of salt solution might act as an adjuvant when symptoms suggested th the extracts were not exerting a sufficient "substitute" action to preve toxic effects of suprarenal insufficiency. The accumulated poisons or "washed out," the extracts might be sufficient for a further period.

SUMMARY

Marked prolongation of the survival period and of the period of good alth was caused in adrenalectomised dogs, by daily intravenous injection Ringer's solution (generally about 100 cc. per kilo of body weight). extrose was almost always added. A table is given showing results on 17 imals. One lived 13 days, 15 hours; one 14 days, 4 hours; one 14 days hours; one 15 days, 5 hours; one 17 days, 6 hours; one 19 days, 8 hours; e 19 days, 12 hours; one 20 days; one 20 days, 3 hours; one 32 days, 17 urs; one 33 days, 5 hours; one 38 days, 2 hours; one 53 days, 16 hours. Marked amelioration of symptoms, even when acute, is almost always oduced during and immediately following injection. Sometimes imals have been rescued, when already comatose, and have lived a long ne in good health. Possible ways in which the injections may act are scussed in the paper.

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STUDIES ON ADRENAL INSUFFICIENCY IN DOGS

V. The Influence of Adrenal Extracts on the Survival Period of Adrenalectomised Dogs[1]

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As soon as we had accumulated a sufficient number of control experiments on animals adrenalectomised but not treated in any manner, we were in a position to test the influence of various measures upon the symptomatology and particularly upon the duration of life and of the period of good health. These, especially the total period of survival after removal of the second adrenal, are definite numbers expressed in days and hours, which can be compared without guesswork with the control tables. At this point it may be well to introduce a few remarks which apply to the comparison of results under imposed conditions (treatments, pregnancy, heat, etc.) with the controls. We believe that the points enumerated must all be taken into consideration if correct conclusions are to be drawn.

In order that the comparison may have any value it is essential that the surgical technique, a difficult one, should be improved to the point at which few, if any, animals die within three to five days of the second adrenalectomy. When the results on animals operated upon so poorly that the fatal result (within a day or two) in most or all of them has been determined by the operation as such the so-called controls can only mislead. Especially is this the case where the writer does not even assemble a sufficient number of controls of his own but relies upon the utterly inadequate data presented in the older literature and in current monographs. Experiments in which death can be legitimately ascribed to adrenal insufficiency can alone be utilised. It is never permissible for an experimenter with an inadequate technique to assume that his operations will be of a uniform degree of badness in a control set of animals and in a set subjected to a certain procedure, the effect of which is to be tested, so that this factor can be considered as eliminating itself. It is only by improving the technique to the point at which the operations are uniformly well done that useful comparisons can be instituted.

[1] A preliminary account of the experiments was published in Science, October 7, 1927, lxvi, no. 1710, 327.

This being given, it is seldom possible to compare averages of the sur-ival periods of two sets of results. Where the number of results is small nd the individual variations large comparison of averages can only lead to rror, as is too well illustrated in literature.

At present no evidence can be so unequivocal as an increase in the sur-ival period because of its definite arithmetical nature. In all our com-arisons of treated animals or of animals in various physiological conditions pregnancy, heat) with controls we have considered it indispensable to lrawing a positive conclusion that this test should be positive.

It is obvious that long survival attributed to treatment or other causes nay be due merely to incomplete removal of the adrenal tissue. The iterature is strewn with instances of this error, not always sufficiently onsidered by the investigators, not always discovered by them but un-loubtedly present. In none of more than 200 dogs operated on by us has ny adrenal remnant ever been found. All the adrenalectomies were otal. Careful exposure of the gland at operation is quite important. Vhen the gland is properly excised and the site of the operation inspected ny remnant can be easily detected and removed. Also it can be seen that he excised gland is intact. Nevertheless, most careful search is always nade *post mortem* for so-called adrenal "rests" or accessories. In one dog a ortion of an adrenal separated from the rest by a constriction was seen nd removed at the operation. In another dog a small accessory adrenal vas seen and removed at operation.

A necessary precaution in a large series of animals subjected to a given reatment is to guard against the possibility that an apparently positive esult may be due to increasing facility in the technique. Therefore, a eries of controls obtained early in the investigation should not be relied ipon exclusively but a new control series should be made while the "treat-nent" observations are going on. We have done this in 36 additional nimals without finding any material change in the results, which are lisplayed in table 2. Additional controls may sometimes be obtained by ising a treatment series, which has clearly yielded a negative result, as a ontrol series. This expedient can only be adopted with great care. As egards the necessary number of animals in a series, no definite rule can, of ourse, be laid down, but an inconsiderable number such as six or seven, s of little use, unless the results are almost uniformly and strikingly posi-ive.

One more point deserves mention, although it will readily occur to nybody who gives even a moderate degree of attention to the matter. None of the results obtained by treatment in our observations can be onsidered optimum. For example, with the Ringer's solution injection he amount of labor involved was far too great, as pointed out in the paper n that subject (paper IV), to permit sufficiently large series to be as-

sembled with varying doses, varying intervals between the injections ar varying composition of the solution. All that could be done by us was adopt an arbitrary dose of one and the same solution injected always approximately the same interval of time. The same was true of the in jections of adrenal extracts. It can therefore be confidently assume that our results, although quite strikingly positive, are not the be which can be looked for.

This paper is devoted principally to the question whether evidence obtainable that the adrenal (cortex) contains a substance which aft extraction can be introduced into animals deprived of their adrenals an can prolong life and the period of good health beyond the maximum valu in the control series. It will be seen when table 1 and some typical pr tocols are studied that the answer to this question is unequivocal; a no inconsiderable number of the treated animals do survive far beyond any thing seen in untreated controls. The period of good health appears to b correspondingly prolonged, perhaps relatively even more than the tota survival period. Not seldom the animal continues to eat almost up to th end, so that it may be impossible to notice the onset of anorexia as definite event presaging death and preceding it. This is usual in the con trols some days before death. It must be repeated that at present we d not deduce a positive conclusion from a *modification* of the symptomatology although we believe this may be observed, unless the total survival perio is increased without question beyond the limits seen in the controls. W have also gained the impression that the pathological changes in the gastro intestinal mucosa are modified by treatment with extracts, hemorrhagi congestion in the mucosa being less common and less extensive. Th congestion of the pancreas, however, is not affected. We have already (Stewart and Rogoff, 1925; Rogoff and Stewart, 1926) developed a hypoth esis of the significance of the morbid changes in the alimentary canal and have found some evidence in support of that hypothesis in the modificatio of the changes by Ringer's solution injections. Whatever their relation to the lack of adrenal function, it is significant that the symptoms and patho- logy should be capable of being modified by treatment. This is the case even in animals which do not outlive controls. As already pointed out (Rogoff and Stewart, 1927) the power to keep the treated animals alive, decidedly beyond the maximum period for controls, is a very severe test since every indispensable factor which the adrenals supply to healthy existence must be substituted for by the gland extracts, whereas the substitution of only one factor might suffice for the prevention of an important but not necessarily in itself a fatal symptom, such as anorexia.

A little more attention may be profitably given to table 1. It contains 29 animals, 20 males and 9 females, non-pregnant and, so far as could be determined by careful observation, not in heat. Since we have shown that

hese conditions tend to prolong life beyond the limits seen in the controls, ales alone are employed as far as possible.

TABLE 1

Animals treated with extracts of dogs' adrenals

UMBER OF NIMAL	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED		BEGAN TO REFUSE FOOD	ALIMENTARY CANAL		PANCREAS CONGESTION	NUMBER OF EXTRACT
	First operation	Second operation					Blood	Congestion		
	kgm.	*kgm.*	*days*	*days*	*hours*	*days*				
123–0	9.0	9.0	12	13	13	13	++	++	+++	II
123–1	7.4	7.7	13	$21\frac{2}{3}$			0	++	+++	II, V
123–3	10.0	9.7	13	$17\frac{2}{3}$		17	0	0	++++	II, V
123–6	9.0	8.8	15	9	6	9	+++	++	++	V
123–7	9.6	9.2	22	$10\frac{3}{4}$		9	++	++	++++	V
123–9	6.5	6.2	24	6	$21\frac{1}{2}$	5	++	+++	+++	V
124–1	10.6	10.4	21	$78\frac{2}{3}$		$76\frac{1}{2}$	0	0	0	V, XII
124–2	10.6	9.7	24	6	22	5	++	++	++	VI
124–5	8.5	9.4	69	12	2	11	0	++	++++	XV
124–7	9.5	9.6	64	9	4	8	++++	+++	+++	XV
124–9	9.3	9.5	12	$10\frac{1}{2}$		10	++	++	+++	XII
125–2	8.6	8.4	11	$7\frac{1}{2}$		$6\frac{1}{2}$	+	+	++++	XII
125–3	7.8	7.3	13	$5\frac{2}{3}$			+−	+−	+	XVI
125–5	10.0	9.6	35	10	23	$9\frac{1}{2}$	+++	+++	++++	XIII
125–7	7.0	7.2	38	$27\frac{2}{3}$		27	0	+−	+++	XVI
126–1	13.5	12.2	41	8	$21\frac{1}{4}$		++++	++++	+	XIV
126–6	10.8	10.2	42	$9\frac{2}{3}$		8	++	++	+++	XV
126–7	11.1	10.2	19	7	$1\frac{1}{2}$	6	+++	++++	++++	XIV
126–8	10.9	10.2	20	$12\frac{2}{3}$		11	+++	+++	++++	XV
126–9	9.7	9.3	20	12	$\frac{1}{2}$	11	0	+	++++	XV
127–3	12.1	11.3	14	11	4	$9\frac{1}{2}$	+++	+++	+++	XVII
127–4	8.9	8.1	15	$7\frac{2}{3}$		7	+++	+++	+++	XII*
127–6	10.9	10.2	17	15	22	15	++	++	+++	XVIII
127–9	10.8	10.2	19	$19\frac{3}{4}$		19	+++	++	++++	XIX
128–0	11.8	11.3	18	$22\frac{2}{3}$		$21\frac{1}{2}$	++	++	++++	XIX
128–3	7.8	7.4	13	$9\frac{1}{2}$		7	+++	++	++++	XIX
129–1	15.3	15.5	16	$11\frac{2}{3}$		$10\frac{1}{2}$	+++	+++	++	XXI
129–3	14.0	14.6	14	$7\frac{2}{3}$		4	+++	+++	+++	XXI
129–7	11.8	11.4	13	5	20	4	++++	+++	+++	XXI

All the dogs were males except 123–6, 123–9, 124–2, 124–5, 124–7, 125–3, 125–7, 26–6 and 127–6.

* Old material.

The occasional rapid improvement after extract injections with the estoration of the animal to a further period of health is sometimes observed lthough usually less striking than in the series treated by Ringer's solution.

TABLE 2

Additional control adrenalectomised dogs

NUMBER OF ANIMAL	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED		BEGAN TO REFUSE FOOD	ALIMENTARY CANAL		PANCREAS CONGESTION
	First operation	Second operation					Blood	Congestion	
	kgm.	*kgm.*	*days*	*days*	*hours*	*days*			
125–4	9.1	9.1	11	$11\frac{3}{4}$		10	+++	+++	++++
125–6	5.8	6.5	23	$8\frac{1}{2}$		7	++	+	+
125–8	7.4	7.3	41	$8\frac{1}{2}$		6			
125–9	9.2	8.3	53	$15\frac{3}{4}$		14	++++	++++	++++
126–0	11.1	9.7	53	$6\frac{1}{2}$		5	+++	+++	++++
121–1	7.9	7.8	14	11	$5\frac{1}{2}$	10	0	0	++
126–2*	11.7	9.3	56	5	$4\frac{1}{2}$	4	++	++	+++
126–3	10.0	10.0	40	$12\frac{1}{2}$		11	++	++	+++
127–0	11.3	11.2	20	9	7	8	+++	++	++++
127–7	10.9	8.5	42	$12\frac{1}{2}$		10	0	0	+++
128–2	12.2	11.7	15	$14\frac{2}{3}$		13	0	+	+
128–9	9.1	9.8	37	15	17	12	++	+++	+
129–4	8.6	10.6	35	4	20	$4\frac{1}{2}$	+	+	+++
129–8	9.4	10.8	29	11	23	10	+++	+++	+++
129–9	7.0	7.5	50	8	22	7	+	0	++
130–0	9.6	9.9	32	6	$4\frac{1}{2}$	5	++	++	++++
130–1	7.1	9.6	55	8	23	7	++++	++++	++++
130–2	6.9	8.7	55	$9\frac{1}{2}$		7	++++	++++	++++
130–3	7.2	7.9	16	$8\frac{3}{4}$		7	+++	+++	++
130–4	6.3	7.0	30	$6\frac{1}{3}$		4	0	+	+++
130–8	6.2	6.1	19	$6\frac{1}{2}$		3–4	0	+	++
130–9	11.4	12.1	21	15	$17\frac{3}{4}$	13	+++	+++	++
131–0	9.9	10.4	21	11			0	0	+++
131–1†	9.3	9.9	16	4	20	3	0	0	++
131–3	13.0	13.0	16	$13\frac{1}{2}$		12	+	+	++++
121–3	9.1	11.0	32	4	$2\frac{1}{2}$	3	++	++	++++
121–4	9.5	8.2	18	5	9	4	+	++	++++
121–5	5.7	5.8	15	11	15	9	+++	++	++++
121–6	7.5	8.2	30	12	16	10		+	+++
122–2	9.3	9.2	21	12	$6\frac{1}{2}$	11	++++	++++	++++
122–5	9.5	9.2	15	12	4		+++	+	++++
123–2	6.7	6.4	13	5	8	4		++	++++
123–4	8.7	8.1	13	7	$10\frac{1}{2}$	5	+++	+++	++
129–0	13.1	13.1	20	$9\frac{1}{2}$		8	+++	+	+++
131–4	14.5	14.0	15	$6\frac{5}{6}$		5	++	+++	++++
131–6	7.5	8.1	15	9	$3\frac{1}{2}$	7	+++	+++	+++

* Had weakness and tremors in muscles of legs and neck before and after operations.

† Very mangy before and after operations.

These effects are not to be neglected in deciding whether an extract is potent or not. Their occurrence will be illustrated in some of the protocols reproduced.

A more detailed examination of table 1 is now necessary. The 29 imals therein contained represent all that received injections of adrenal tracts with the exception of certain cases in which complications not nnected with the treatment occurred. These will be enumerated but are cluded from the table. The material for injection was prepared, by rious methods, from fresh dogs' adrenal glands obtained aseptically om animals that were being adrenalectomised, and was kept in the refriger- or. No material kept more than a week to ten days was used. As tracts of the whole gland were employed the medulla contributed some- ing to the extracts and adrenalin was always present but in small amounts s tested colorimetrically). On standing the small adrenalin content came still less. We have no hesitation, however, in concluding that the sults were not due in any important degree to adrenalin. For 1, our periments on rabbits and cats instituted 10 years ago showed that in imals which had developed the serious symptoms associated with adre- l insufficiency, only a transient improvement could be effected by intra- nous injections of adrenalin. Other investigators have also found that e could not be prolonged materially in this way. 2, Control experiments dogs with injection of quantities of adrenalin as great as or greater than uld have been contained in the extracts yielded negative results. Three these experiments have been included as additional controls in table 2.

In another series, extract injection experiments in which slaughterhouse aterial (sheeps' adrenals) was employed, the cortex was separated from e medulla and extracts of cortex were made. These experiments will be alt with in a later paper. It will suffice to state here that the results were sentially similar to those in table 1. The symptoms and morbid changes the gastro-intestinal tract appeared to be influenced still more favorably an in the series with dog's adrenal extracts, and life was prolonged in ite as large a proportion of the animals well beyond the extreme limits en in controls; while the proportion of animals which without surpassing e maximum of the controls, that reached the higher levels of the survival riod, was increased.

The dose of extract usually employed was 0.5 cc. or 1 cc. intravenously ministered.

Seven dogs in table 1 out of 29 may be considered as surpassing the aximum survival period shown in the controls (tables 1 and 2, paper I), e only slightly (nearly 16 days, animal 127–6). One animal lived $17\frac{2}{3}$ ys; one, $19\frac{3}{4}$ days; one, $21\frac{2}{3}$ days; one, $22\frac{2}{3}$ days; one, $27\frac{2}{3}$ days; and one, $\frac{2}{3}$ days. No such numbers are to be seen among the controls. There is circumstance in the injection experiments to which they can be attrib- ed except the presence in the adrenal extracts of something which for a ne can "substitute" for the specific substance produced by the adrenal ortex). It is quite immaterial whether this substance acts by aiding in

the destruction or neutralisation of a poison or poisons produced in tl absence of the adrenals, or supplies something necessary to the continue normal functioning of essential organs.

The question has already been alluded to (Rogoff and Stewart, 192' why some of the animals and not all are favorably influenced. It cann be stated at present that even with a potent, stable preparation whic could be kept without deterioration for a time sufficient to permit large series of animals to be tested, all of the animals would be benefited, equally benefited. There may be individuals more susceptible than othe to the beneficial effects of extracts. Some may be more capable of storir the important substance or of using it economically than others. A present it is not profitable to speculate further upon this matter.

From the way in which our experiments were performed, it is impossib that great variations should not have occurred in the effects of the extract In the first place, the extracts were obtained from the adrenals of differer dogs. It could hardly be expected that different extracts made by tl same method from identical material should necessarily be equally poten The extracts were kept in the refrigerator and were used as long as the remained clear. About a dozen different extracts (all made by the sam technique) were employed more or less in treating the series of dogs i table 1. Only a small number of animals could be treated at one tim If an extract seemed to be more successful than usual it could still be ac ministered only to a small number of animals. Nor was there any way c knowing how rapidly a given extract, initially potent, lost potency o standing, even if it remained clear. Of the extracts mentioned in table] II seemed to be specially successful. At least the three animals (123-(123-1, and 123-3 in the table), in which it was injected, all showed lon survival periods, two of them well beyond the maximum of controls. Bu another extract (V) was employed in two of these animals in the latte part of the experiment. Extract XIX seemed to be unusually potent, a two out of the three dogs in table 1, in which it was employed, lived muc beyond the maximum seen in controls.

In the second place injurious substances may have been introduced int the blood as well as the beneficial substance, and the amount of these woulc of course, vary in the different extracts and probably in the same extrac when kept. Control tests on unoperated dogs were made in order to se whether any effects resulted which could be attributed to such injuriou substances, but the test was negative.

Third, it has already been seen in paper IV (Rogoff and Stewart, 1928 that even with the same injection liquid (Ringer's solution) great variation occur in the effects in different dogs.

Undoubtedly, had the series been extended an additional number of lon survivors could have been added to the table. But there would have bee

o point in spending a large amount of time for this purpose. Our object as achieved when it was demonstrated clearly that extracts were obinable which could be safely administered (intravenously) to dogs and hich could "substitute" for the glands. We deprecate naming every tive substance obtainable from an endocrine gland a "hormone". That ould only be done when it is known to be given off physiologically from e gland. Very few of the active substances which have been separated om endocrine glands can at present satisfy this condition. Nevertheless, ere is no harm, and a certain convenience, now that it is known that tive extracts can be derived from the adrenal (cortex), in giving a name the hypothetical body. We considered "corticin," but rejected it. It ggests, of course, a substance obtainable from cortex but does not indicate hat cortex; it might be kidney or brain. The best term, in our opinion, "interrenalin," a substance derived from the interrenal gland tissue. We ve always considered "adrenin" a physiological misnomer, and the me would be true of "interrenin," although a syllable would be saved.

The proof that a substance can be extracted from the adrenal (cortex) ich can prolong life after adrenalectomy has now been given. This afrds an indispensable basis for all work directed to isolate and purify the bstance. Till this proof was forthcoming work in this direction could t be pursued with confidence or any promise of success.

A few of the protocols will now be given in condensed form.

Dog. Male. Record number 123-0. On October 28, 1926, bodyweight 9.0 kgm.; t adrenal removed (0.70 gram). November 9, weight 9.0 kgm.; right adrenal reved at 10:30 a.m. (0.72 gram). November 10 to 12 excellent condition. On vember 11 received 1 cc. of extract II, at 3:00 p.m. Large appetite. November in the morning, hind legs somewhat wabbly; getting more apathetic; emesis utral to litmus); refused all food today. At 3:00 p.m., received 1 cc. of extract II. e heart was slowed then accelerated after the injection. At 4:15 p.m., more esis of frothy matter (neutral); steady on legs but apathetic. November 14, at 0 p.m., much better; took food readily. November 15, very good condition, but led twice as if alarmed. At noon took a good meal (meat). At 12:30 p.m., reved 0.5 cc. of extract II. In the evening violent yelling and barking fit, termind by his eating biscuit. November 16, refused all food; more apathetic again. vember 17, at noon ate a good meal (meat). In much better condition; pugnaus. Was ready for more food in the evening. November 18, condition very good; well. At 10:00 p.m., received 0.5 cc. of extract II. On November 19 and 20, ained in good health; appetite good; pugnacious. November 21, unchanged; eived 0.5 cc. of extract II. Defecated normally. November 22, slight diarrhea blood). Refused food; quite apathetic. At 5:00 p.m., vomited yellow liquid id to litmus). At 11:30 p.m., just died; had not changed his position.

Autopsy. Pancreas considerably congested. Stomach contained bile and blood; cosa hemorrhagic. Duodenum contained bile-stained mucus; mucosa moderly congested. Small intestine moderately congested; only a little blood in lumen.

A feature in the history of this animal, after the second adrenalectom was the fluctuation in the state of health and appetite. The impressi was given that the animal was being picked up from time to time (by t injection?). The terminal stage was very brief. The number in the tal in the column headed "began to refuse food" refers to the complete anore on the last day. There had been several occasions on which no food w taken for a day, but appetite was regained.

Dog. Male. Record number 123-1. This animal was treated with extract at the same time as dog 123-0. October 28, 1926, weight 7.4 kgm.; left adrenal cised (0.70 gram). November 10, weight 7.65 kgm.; right adrenal excised at 10 a.m. (0.80 gram). November 11, excellent condition. Received 1 cc. of extract The same dose was given on November 13 at 3:00 p.m. Remained in good health November 14. On Novemver 15, after injection of 0.5 cc. of extract II, he yell and ran about in the cage, but ate biscuit readily. November 16, had a short "ye ing" spell in the morning but ate well. November 17, ate meat readily, chewing t bones; condition excellent; normal solid stools. November 18, condition goo took food well; pugnacious; 0.5 cc. of extract II. November 19 to 21, unchange On November 21, received 0.5 cc. of extract II. November 22 to 24, condition mained excellent; appetite keen; injected with 0.5 cc. of extract V (a new lot). N vember 25 to 26, unchanged; appetite good. Weight 7.6 kgm. November 27, co dition seems very good; eating well, but hind legs seemed somewhat stiff; slig muscular twitching but no weakness. Received 0.5 cc. of extract V. November condition excellent; marked pugnacity. November 29, less energetic; but ate good meal. At 4:00 p.m., received 0.5 cc. of extract V. November 30, lies abo more than usual; more apathetic; but eating well. Can run well, although somewh stiff at first. December 1, seemed more lively than yesterday. Ate a fair me (meat) at noon. At 1:00 p.m., injected with 0.5 cc. of extract V. He walked we December 2, died early this morning. The autopsy showed moderate congestion portions of gastro-intestinal tract but no blood in the lumen. Pancreas considerab congested.

The final stage was even shorter than in dog 123-0; in fact it cannot said that there was any definite stage characterized by anorexia. In d 123-3 also, this stage was very brief. In a number of the injection expe ments we received the impression that the animals artificially kept ali beyond the maximum survival period of the controls, were liable to collap more suddenly than the latter. Possibly an increase in the number injections at this point might have caused renewed benefit, but this was n done as it seemed preferable first to finish the series according to the pr arranged plan.

Dog. Male. Record number 123-3. October 29, 1926, weight 10.0 kgm.; rig adrenal removed (0.70 gram). November 9, weight 9.65 kgm. November 11, weig 9.65 kgm.; at 10:00 a.m., left adrenal removed (0.80 gram). November 12, very go condition; took food, but soon vomited. Received 1 cc. of extract II. Novemb 13, good condition. November 14, took food but some emesis (bilious). Novemb 15 and 16, excellent condition; eating well. On November 15, 0.5 cc. of extract I November 17 to 21, unchanged. On November 21, received 0.5 cc. of extract I

ovember 22 to 25, unchanged; good appetite. On November 24, received 0.5 cc. of tract V (new lot). November 26, weight 9.2 kgm. Good condition, but wabbles little at first in walking. Less energetic. Fair appetite; some emesis. Received 5 cc. of extract V. November 27, quite shaky and more apathetic; took fair meal noon. At 6:00 p.m., weak. November 28, better than yesterday, but refused all od; hind legs weak. Received 0.5 cc. of extract V. November 29, died during the ght.

Dog. Female. Record number 125-7. December 8, 1926, right adrenal excised; ight 6.95 kgm. The weight was taken many times up to February 13, 1927 and ly varied slightly from 7 kgm. February 15, left adrenal excised at 10:00 a.m.; ight 7.2 kgm. February 16, condition very good; ate well. Injected with 0.5 cc. extract XVI. This was used throughout in the same dose. Weight 6.7 kgm. bruary 17, unchanged. February 18, had yelling and racing fits all day but e well. Injection. February 19, some yelling, but ameliorated. Ate well. bruary 20, seems well, but did not eat much of meal (bread and milk). Injection. om February 21 to March 3, remained in excellent health, eating (bread and milk, eat) well. Injected on alternate days. Weights 7.25, 7.2 kgm. March 4, appe- e poor, but finished the meal slowly. Injected. March 5, good condition, but e more slowly. March 6, unchanged. Still eats (meat, especially), but appe- e is not so good. Injected. March 7, weight 6.9 kgm. Fairly active. Emesis ilious) in forenoon, but ate noon meal (meat). Up to March 13, no change. Con- ues to eat meat well but since March 11 has not cared for biscuit which she liked eviously. Injections as usual, the last being on afternoon of March 12. March 14, bbles in attempting to walk; apathetic; appetite small but was coaxed into eating me ham (not retained). Prefers not to get up, but no acute symptoms. Weight 5 kgm. March 15, dead in the morning. There was practically no congestion of stro-intestinal tract. There was no evidence during life that the animal was either heat or pregnant and this was confirmed at the autopsy.

Dog. Male. Record number 127–9. February 17, 1927, left adrenal removed; ight 10.2 kgm. February 21, weight 10.5 kgm. February 28, weight 10.1 kgm. rch 8, right adrenal removed; weight 10.2 kgm. The animal remained in excellent ndition till March 18. On alternate days received 0.5 cc. of a fresh extract (XIX), ginning March 9. On March 19, seems somewhat less lively; slightly unsteady on nd legs. Ate biscuit readily; in the evening seemed normal. Up to March 24, change. Hair coming out; does not care for biscuit any more but takes meat. rch 25, not quite so active as heretofore, but eats (meat), although with dimin- ed appetite. Injections continued as before. March 26, eats meat, but appetite sened; more apathetic; does not wish to leave the cage. March 27, refuses all food; ssing bloody mucus from anus; very apathetic. Walks well when he does get up. 3:00 p.m., received the last injection. On the morning of March 28, he was dead.

Dog. Male. Record number 128–0. February 18, 1927, left adrenal removed; ight 11.8 kgm. Up till March 7, the weight remained about 11 kgm. March 8, ht adrenal removed at 10:30 a.m.; weight 11.3 kgm. Till March 15, the condition the animal was very good; appetite excellent. Received injection (0.5 cc.) of ract XIX on alternate days throughout the whole period of survival. Seems a bit f" but ate well. Somewhat unsteady on hind legs. March 17 to 19, very good ndition; eating well. Injection continued on alternate days. March 20, less alert. fused biscuit, which he always took before. Ate meal of bread and milk, but later nited it (with bile). March 21, apathetic. Refused food till coaxed. Weight

11.35 kgm. Unwilling to get up, but walks all right. March 22 to 24, unchange Eats meat but not with great appetite. Refused bread and milk. Less vigorou than a few days ago. March 25 to 28, more alert; eats meat with good appetite. Is little stiff in walking. A little blood in stools. March 30, lacks strength in hind leg wabbles when walking. No food taken today. More apathetic. Some emesis (bile At 3:00 p.m. and 9:00 p.m. passed some blood from bowel. March 31, at 2:00 a.m. losing consciousness. Died before 6:00 a.m.

Dog. Male. Record number 124–1. November 5, 1926, weight 10.55 kgm.; rig adrenal excised. November 9, weight 10.3 kgm. November 26, weight 10.4 kgm left adrenal excised at 11:00 a.m. November 27, good condition; ate well. R ceived 0.5 cc. of extract V. November 28 to December 4, very good condition; eatin well. Received on alternate days 0.5 cc. of extract V. December 5, ate only a litt (bread and milk), otherwise unchanged. Received 0.5 cc. of extract V. Decemb 6, some shivering, which passed off. Ate well. December 7 and 8, excellent cond tion. Received 0.5 cc. of extract V on each of these days. December 9 to 15, co dition remained excellent; appetite very good. On alternate days received 0.5 c of extract V. On December 9, weight 10.5 kgm. On December 16 finished extract henceforth he got extract XII on alternate days (0.5 cc. to 0.75 cc.) till December 2 when the injections were discontinued. Weight December 23, 10.85 kgm. H condition remained good. December 23, 1926 to January 2, 1927, no change see He begs as usual standing on hind legs, and eats ravenously. On December 3 weight 10.55 kgm. On January 2, less active; some apathy; and hind legs weaker b ate fairly well. January 3, distinctly more apathetic and unsteady in walking; ea meat, but with less appetite than usual and neglects the bones. Lies about mo than usual. Some emesis (bile) this forenoon. At 1:30 p.m., injection (1.25 cc. extract XII). At 3:00 p.m., ate meat and seemed improved. At 4:30 p.m., decided improved, walking on hind legs; not at all wabbling and much more alert. The i jection seems to have picked him up completely. On January 4, he was normal an eating well; continued in excellent condition till February 6. Normal behavio towards female dogs. Weights on January 10, 10.2 kgm.; on January 17, 10.1 kgm on January 24, 10.4 kgm.; on January 31, 10.15 kgm.

From February 6 onwards, he became quieter, his appetite became less kee although he continued to eat meat. On February 7, weight 9.85 kgm. On Februar 10, not much change. On February 11, he was decidedly below par. Apathetic, b not weak. February 12, refused meat at noon, but was coaxed to eat a few of the be morsels. Apathy increased. A little matter in the corner of the eyes (often seen last stages); head hanging down; skin of cheeks and jaws sags as is often seen whe asthenia is developing. At 5:30 p.m., he seemed more alert; walked about more an was not in the least wabbly. February 13, found dead at 9:30 a.m. He must hav died early in the morning. The most striking feature in the post-mortem appea ances was the complete absence of congestion in the gastro-intestinal tract or of bloo in the lumen, as indicated in the table. Another feature was the great size of th parathyroids. We simply note this without attempting to explain it.

One or two points in the protocol require some discussion. When injec tion was intermitted the animal continued in good health for about 10 days Was he then really being kept alive by the injections? The only answe but in our opinion a sufficient one, is that there is nothing else to which th long survival can be attributed. At the operations the adrenals were see

ɔ be completely removed. The completeness of the removal was care- ılly verified at autopsy. No adrenal accessory was discovered by most areful search. This is true of all the dogs both the control animals and ıe animals treated by injections of Ringer's solution, of adrenal extracts nd in other ways. To suppose that in those 29 dogs of table 1 we acci- entally hit upon 6 or 7 which lived distinctly longer (in some cases very ıuch longer) than the maximum seen in nearly 150 controls is not reason- ble. That an animal being kept alive by injection of extracts should live 0 days after intermission of the treatment before showing characteristic gns of deterioration is not at all improbable. For that is about the time hen many dogs begin to develop the fatal symptoms after adrenalectomy. the capacity of many dogs to live in good health as long as that after emoval of the adrenals is due to active substance stored elsewhere than in ıe adrenals (possibly but not necessarily in the genetically similar tissues f the sex glands), it may be that the active substance in the extracts is lso stored. If there is not an actual storage of the active substance which ıables the animal to go on living for a time after injections have been iscontinued, there may be a more or less durable change in certain func- onal activities due to the extracts and outlasting their administration. r, if the survival of controls for a time is due to the slowness with which ıe intoxication increases to the point at which definite symptoms appear, ıen the same thing might be expected to happen on intermission of in- ection of extracts. The prompt recovery of the animal from an obviously erious condition when the administration of extract was resumed corrob- rates the conclusion that his survival is to be attributed to the extract. hat he should live more than a month after the last injection could not ave been anticipated. We see no reason to doubt that this was due also ɔ the previous injections. He was not being kept alive by adrenal tissue r none was found, as already mentioned, and he died at last and with ttle, if any, warning, with symptoms characteristic of adrenal insuffi- ency. He would be as much out of place among the controls, with a ırvival period of 30 to 40 days, as with the full period of nearly 80 days. he only new factor is the adrenal extract. There the matter must be left t present. The sudden end is worthy of note, as is the fact that some food as taken almost up to death. The complete absence of the common athological changes in the gastro-intestinal tract is a feature which has een already mentioned.

Six animals which received injection of extracts are not included in the ıble because of certain complications. Three were pregnant, and lived 7 days, 4 hours; 24½ days and 33½ days respectively. One animal had een used before the first adrenalectomy for testing any possible harmful fects of extracts. It was also in quarantine on account of mange and spiratory trouble. This animal lived 7 days, 19½ hours. One, very

mangy before the first adrenalectomy and remained so after the secon operation, lived 5 days, 15 hours. In another dog, which lived 5 days, 4 hours, the right adrenal was very deep seated under the liver; the cava wa torn, necessitating its repair, and involving much more trauma than usua

As illustrating the immediate beneficial effect of transfusion of adren vein blood from one normal dog into a second (adrenalectomised) dog, protocol may be given. We do not think that blood from the adrenals different from blood from any other source in this respect and we have see ordinary defibrinated blood produce a similar effect. It is quite unknow whether the substance which has life-prolonging power is given off to th blood. The epinephrin always contained in blood from the adrenals migh or might not have a temporary beneficial influence, even in the sma concentration normally present. But all the evidence goes to show tha adrenalin injections do not materially prolong life. Many of the exper ments of Tournade on anastomosis of the adrenal vein of one animal wit the jugular of another show nothing more than has been known for considerable time, that epinephrin is given off from the adrenals.

Dog. Female. Record number 85–9. September 15, 1924, right adrenal excise September 22, left adrenal excised. Took food up to September 25, when she refuse it; was weak though able to walk. Temperature 36.9°C. September 26, very a thenic. Temperature 33.4°C. At 3:30 to 4:00 p.m., made a cava pocket in a larg (normal) dog and united the pocket by cannula with the jugular vein of dog 85– Transfused 10 to 15 minutes. Then collected blood from the pocket and after d fibrination injected it into dog 85–9 (100 cc.) at 4:30 to 5:00 p.m. At 5:05 p.m., th temperature was 37.8°C; the dog defecated (watery stool) and drank water. Able t stand better. At 9:00 p.m., temperature 34°C. A mixture of Ringer's solution an adrenal blood (100 to 125 cc.) was injected. Respiration and pulse rate increase Continued to urinate freely. From 10:00 to 10:40 p.m., she began to walk abou Temperature 37.1°C. At 10:50 p.m., injected 800 cc. Ringer's solution with de trose to make up 2.5 per cent. Free urination. At 11:20 p.m., temperature 37.8°C At 11:35 p.m., took a run in corridor. No weakness in legs. September 27, die early this morning.

As soon as it appeared that positive results were being obtained, wit injection of extracts of dogs' adrenals, that is, survival periods lengthene beyond the maximum seen in the controls, it was decided to accumulat another series of controls (untreated adrenalectomised animals, either male or females, non-pregnant and not in heat). Only in this way could the pos sibility be excluded that increased practice in the operation with improve ments in the technique might be responsible for longer survival, which w might be attributing erroneously to the extracts. Table 2 displays th results of 36 new controls. It will be seen that they are essentially th same as those in tables 1 and 2 (paper I). The dogs were males excep numbers 121-1, 121-3 to 121-6 (inclusive), 122-2, 122-5, 123-2, 123-4, 125- to 126-0 (inclusive), and 128-2. Included in the table are 8 dogs (121-3 t

.21-6, inclusive, 122-2, 122-5, 123-2 and 123-4), which were injected with a ommercial preparation of corpus luteum. It will be seen from the conlensed protocol of one of these dogs (122-2), which survived 12 days, $6\frac{1}{2}$ ιours, that the results were negative, no noticeable effects of any kind beng produced. The experiments were, therefore, considered as additional ontrols.

Dog. Female. Record number 122-2. October 6, 1926, weight 9.3 kgm.; right drenal excised. October 27, weight 9.2 kgm.; left adrenal excised at 10:00 a.m.)ctober 28, good condition; 1 cc. commercial corpus luteum extract injected inravenously. October 29, had two yelling spells; injected 1cc. corpus luteum extract.)ctober 30, excellent condition; ate well though she had a yelling spell during the ıeal; injected 1 cc. corpus luteum extract. From October 31 to November 6, she ontinued in very good condition, eating well and behaving normally except that on wo or three occasions she had hallucinations; 1 cc. of corpus luteum extract was dministered, intravenously, daily. November 7, emesis (bile); active but not keen or food (bread and milk; dog biscuit); in the evening she appeared somewhat apahetic and stretched a good deal. November 8, total anorexia; emesis (bilious; lkaline to litmus); asthenic; at 2:00 p.m., very asthenic; diarrhea (bloody stool); :00 p.m., comatose; died at 4:30 p.m. *Autopsy.* Liver, spleen and kidneys conested, pancreas very markedly congested. Uterus small (virgin); ovaries small nd no visible corpora lutea. Stomach contained very bloody liquid; the mucosa ras hemorrhagic and three small ulcers, extending through to peritoneum were resent in the pyloric end. In the small intestine the contents were bloody throughut, increasingly so downwards; the entire mucous membrane was exceedingly emorrhagic. The large intestine and rectum were empty; mucosa moderately ongested.

Three animals (129-0, 131-4 and 131-6) received intravenous injections of drenalin in amounts greater than could have been present in the adrenal exracts. The results were also considered negative and the animals included mong the controls. One male dog (122-4) belonging to a group intended for ıjections was observed to be particularly vigorous and gave the impression hat he might live a long time. He was, therefore, allowed to live without reatment, as a control. The survival period was the longest we have yet een in untreated dogs, namely, 16 days and 6 hours. The animal is not ıcluded in the table because it seemed to us that if that were done the rest f the group to which he belonged ought to go in also, which was impossible. 'he matter is of no consequence, but it is clearly demonstrated that a dog eceiving no treatment can survive the removal of the second adrenal as ɔng as $16\frac{1}{4}$ days. This is the maximum period seen in our controls, at least 120).

SUMMARY

Proof is given that extracts of adrenal cortex can prolong the period of urvival of dogs after adrenalectomy, beyond the maximum seen in control, ntreated animals.

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STUDIES ON ADRENAL INSUFFICIENCY

VI. The Influence of "Heat" on the Survival Period of Do after Adrenalectomy

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We have already stated (Rogoff and Stewart, 1927) that the conditi of heat enables the totally adrenalectomised bitch to survive much beyo the maximum period seen in control male dogs or females not in heat a non-pregnant. Data on one animal (record number 120–5) have be previously referred to (Rogoff and Dominguez, 1927). The effect of a renalectomy on the blood pressure of the animal was being studied by t carotid loop method. The second adrenal was removed towards the e of oestrus. The animal survived about 36⅔ days. The blood pressu was not affected by the condition of heat. Another bitch (121–9), in whi the second adrenal was removed during heat, survived 32 days.

Condensed protocol. Dog. Record number 121–9. October 5, 1926, weig 9.1 kgm. Right adrenal (0.60 gm.) removed. October 15, 1926, weight 8.5 kg Left adrenal (0.62 gm.) removed at 10:30 a.m. Post-operative recovery excelle Up to and including November 11, 1926, the animal remained in excellent healt eating well and very lively. On November 12 she seemed somewhat less lively a although she ate a good meal (meat) her appetite was not as keen as before. Refus biscuit absolutely. November 13, distinctly less alert than heretofore but not all asthenic. Reluctantly ate a portion of meal (meat) and some rabbit. Novemb 14, some bilious vomit. Refused food completely. Apathetic. November 1 getting more apathetic. Total anorexia. Perfectly intelligent. Somnole November 16, at 10:10 a.m., died without a struggle. Autopsy at once. Pancre markedly congested. Stomach and whole of small intestine contained blood (a bile). The mucosa was congested extensively in the stomach and less so (in patche in the small intestine. Uterus, non-pregnant, hypertrophic. Ovaries congeste numerous corpora lutea visible on surface.

Nothing in this protocol calls for special remark. The survival peri was of course much beyond anything seen in the very extensive series controls (at the time of writing, about 150). This is true of all of the fo animals included in the paper. The period of good health extended, as the great majority of the controls, up to 2 or 3 days before death. Acu symptoms apart from anorexia and slight emesis were absent. The sudde

hange for the worse between the last night and morning with no acute ymptoms, not even coma, is unusual.

The third dog (124–0) had the first adrenal removed as soon as she was bserved to be in heat, while the bloody discharge was going on. Eleven ays later, the second adrenal was excised. The fact that the beginning f the pro-oestrum is marked in dogs by this discharge renders them specially iitable for such experiments as those described. In dog 124–0 the second drenalectomy was performed before heat was over but towards the end of ;. The prolongation of life and of the period of health although not as reat in dog 124–0 as in other dogs, was nevertheless quite distinct. The nimal survived the loss of the second adrenal $21\frac{1}{2}$ days, and remained in ood health and eating well for about 19 days.

Condensed protocol. Dog. Record number 124–0. November 5, 1926, weight 25 kgm. Right adrenal removed. November 9, weight 9.1 kgm. November 16, eight 9.35 kgm. Left adrenal removed at 10:15 a.m. Excellent recovery. No-ember 17, condition excellent. November 18 and 19, took some food but not much. ovember 20 and 21, appetite improved. November 22 to December 4, 1926, her ndition was very good. Appetite excellent. December 5, refused food. December total anorexia; rather apathetic; not asthenic. December 7, refused all food; rap-ly failing. At 4:00 p.m., quite asthenic and very somnolent. At 9:00 p.m., com-etely comatose. Pulse slow and feeble; respiration slow and shallow. Passed a nall amount of bloody fecal matter. Died at 10:20 p.m. *Autopsy* at once. Pan-eas greatly congested. Considerable amount of blood in stomach, small and large testines; mucosa of whole gastro-intestinal tract much congested and hemor-agic. Kidneys, liver and spleen not greatly congested. Ovaries somewhat en-rged; many corpora lutea. Uterus large, non-pregnant.

The fourth dog (124–8) was in some ways the most interesting of the ries, particularly in the long survival period, about $64\frac{2}{3}$ days. The second drenal happened to be removed shortly before the beginning of pro-oestrum. he changes accompanying or initiated by heat were sufficient to carry the nimal on for a period longer than the average gestation period in dogs. he nutrition of the animal was well maintained, as shown by the body eights and post mortem condition.

Condensed protocol. Dog (record number 124–8). Weights on November 30, ecember 9, 17, 23, 31, 1926, January 10, 1927, January 17, 24, 31, February 7, 13 and were 11.6, 11.4, 11.3, 11.55, 11.15, 11.1, 11.1, 11.1, 10.8, 10.8, 10.6 and 10.6 kgm. spectively.

November 30, 1926, right adrenal excised. February 15, 1927, left adrenal excised 10:00 a.m. Excellent post-operative recovery. February 16 to 18, ate well, but t very active. February 19, good appetite. February 20, quieter, not eating much. bruary 21, condition excellent. Ate well. She showed signs of heat. Attracted ale dogs. February 23, bloody discharge from vagina was observed. She remained very good condition and eating well till March 7, when she seemed less alert. Re-sed bread and milk but took meat readily. No asthenia but not very active. arch 8 to April 15, remained in good health, eating meat and bread and milk regu-

larly but sometimes refusing biscuit, which previously she ate readily. The weigh on March 14, 21, 29, April 4 and 11 were 9.6, 9.5, 9.75, 9.9 and 10.1 kgm. respectivel April 15, she did not care for bread and milk now but ate only meat. April 1 seemed to be getting less active but ate meat readily. Eyes "mattery" from this tir on. April 17, ate meat rather reluctantly but finished the meal. Decidedly le active. April 18, lay about a good deal. Could only be coaxed to take a few sma pieces of meat. Not weak. April 19, apathetic, but not asthenic. Walked w when coaxed out of cage. Complete anorexia. Slight emesis. April 20, decided more apathetic. Asthenic. 5:30 p.m., heart slow, some emesis (bilious). Refus all food. April 21, died early in the morning.

Autopsy. Fat plentiful in usual situations. Liver, spleen and kidneys not co gested. Pancreas, no congestion whatever. Little, if any, congestion in the gastr intestinal mucosa except in two or three Peyer's patches. No blood anywhere lumen. The fundus of stomach was invaginated into the pylorus. Right ova larger than left, and showing prominent corpora lutea while the left showed no corpo lutea on the surface. Uterus somewhat thickened with endometrium more promine than usual, forming folds. Certainly non-pregnant.

The very long period of survival after the second adrenalectomy is tl most striking feature in this experiment. With the exception of occasion diminution of appetite lasting for a day or two and accompanied by son lessening of activity, the animal for almost the whole period was in goo health. The serious terminal symptoms appeared about 3 days befo death (anorexia, apathy). Decided asthenia was present only about days before death. Emesis was never a prominent symptom. Durii the rest of the period of survival it would have been impossible to disti guish this animal from a normal bitch. She was extremely lively in tl cage and romped around when let out of it, standing up in the cage to gre persons entering the room. During heat she exhibited the usual behavic seeking out males, especially a totally adrenalectomised dog (127–4 whose second adrenal had been removed 3 days before. He responde readily but all possibility of impregnation was definitely excluded. Tl second adrenal was removed 77 days after the first, and about the beginnii of heat. A point of interest is the absence of congestion in the gastr intestinal tract and of blood in the lumen. This, however, is sometim observed in the control dogs. More striking is the absence of congestic in the pancreas, which is rare in the controls.

Discussion. The results obtained on the four animals being unifor as regards the markedly increased survival period and the period of goo health, we do not hesitate to conclude that the changes associated wi heat do in some way compensate for the loss of the adrenals. The peric of survival may even exceed slightly the average gestation period in the do When four animals are seen to survive the removal of the second adren into the 37th day, 32 days, into the 22nd day and into the 65th day r spectively and these constitute the entire number of animals operated c in this condition, it is superfluous to multiply experiments. Nothing lil

hese results are to be found among a series of controls nearly 40 times as ırge. The longest period of survival was in a dog which lost its second drenal about a week before the bloody discharge was observed. In the thers the second adrenal was removed towards the end of oestrus. What he change is which is associated with the increase in the survival period it not possible to say at present. One might think of the changes in the vary connected with ovulation. The marked alterations in the uterine ıucosa are not excluded as a possible factor, nor changes in the interstitial ells of the ovary. At present we have no experimental data on this ques-on. Since we have established the marked influence of heat upon the ırvival period the similar influence of pregnancy (Rogoff and Stewart, 927) must be considered from a new angle. Every pregnant dog, fertilized ı the normal way, passes through a period of heat. Since heat in the bsence of pregnancy is associated with an increase in the survival period, ometimes fully as great as the maximum seen in pregnancy, the question ıay be asked whether the apparent effect of pregnancy upon the survival eriod is not really the influence of heat. It would probably entail a great eal of work to settle this question if a sufficient number of experiments to e treated statistically were to be made. However, as the tissues affected ı heat are for the most part the same as those affected in pregnancy, the ıanges being perhaps carried farther in the latter condition, it would seem robable that pregnancy, as such, has an influence in the same direction as eat. It may be remarked, and we have seen illustrations of this in our ork, that pregnancy may sometimes be an unfavorable factor, adding to ie handicap of loss of the adrenals. This is almost self-evident. The ccident of the initiation of premature labor by the surgical operation itself econd adrenalectomy) has been observed by us. This does not necessa-ly diminish the chances of long survival. In the case of heat the condition oes not add directly to the handicap imposed by adrenal insufficiency alone nce heat causes such slight disturbance, at least in comparison with the rmidable complications which may be introduced by pregnancy. The eneficial changes may be assumed to be present in every case of heat though the time of survival may vary with the position of the second eration in the heat period, and also, it is to be supposed, with the in-vidual dog. The necessity of absolute exclusion of the possibility of ıpregnation is self-evident. In our animals this was done, and it was rified, post mortem, also that they were non-pregnant. The discovery that at increases the survival period renders it necessary to use for controls ly males, or females not in heat and not pregnant. The safest plan is t to use females at all. It is known that none of the females in our ries of controls was pregnant, and it is practically certain that none them were in heat. Only males are now employed by us as con-ols and this has been the case for a long time. Males are also being

used exclusively for studies of the effects of cortical extracts. It is possibl however, that the administration of such extracts from time to time animals in heat (or to pregnant animals) might cause a still greater lengthe ing of the survival period. If this occurred and the difference was marke or if a "picking up" of the animal by extracts after it had begun to decli could be distinctly made out this would also constitute a positive test the efficacy of an extract. The fact must be emphasised that physiologic changes apparently so slight as those connected with heat should be cap ble of counteracting the extremely serious, and indeed uniformly fat effects of total adrenalectomy in dogs. All the indispensable facto formerly supplied by the adrenals must therefore be substituted for by oth tissues or processes. While we make no statement as to the tissues i volved, the suggestion that cells in the ovary, similar in origin and structu to the adrenal cortex are responsible is a plausible one. If these cells (the uterine mucosa) contribute something similar to the active substan of the adrenal cortex during the periods of sexual quiescence, this contrib tion could hardly be expected to be a sensible one as compared with th due to the cortex. Nor can it be assumed without proof that even i heat these structures form or liberate appreciable quantities of the su posed active substance. It is quite possible that the condition of adren insufficiency is a necessary stimulus. In paper III (Rogoff and Stewar 1927) it has been pointed out that metabolic changes associated wit pregnancy, apart from changes in the group of tissues mentioned, may i some way supply deficiencies or neutralise toxic conditions due to the a ence of the adrenals. The same thing may be true of heat. On all the points, however, definite knowledge is entirely lacking.

SUMMARY

"Heat" (in dogs) is associated with a marked lengthening of the surviv period and the period of good health after removal of the adrenals. A the dogs studied gave positive results. One lived into the 22nd day aft excision of the second adrenal; one lived 32 days; one lived into the 37t day, and one into the 65th day. Nothing like these survival periods h been seen in the far more numerous series of control dogs (about 150).

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STUDIES ON ADRENAL INSUFFICIENCY

'II. Further Blood Studies (Cholesterol and Calcium) in Control Adrenalectomised Dogs

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In paper II (table 1) of the series (Rogoff and Stewart, 1926) estimations re given of non-protein nitrogen, urea nitrogen, uric acid, preformed and otal creatinine, amino-acid nitrogen, undetermined fraction of non-rotein nitrogen, Cl and dextrose in the blood of adrenalectomised dogs ot subjected to any treatment. Before and since that time determina-ons of cholesterol and calcium were made by us. The non-protein nitro-en showed a marked rise when the characteristic symptoms, especially efusal of food, developed. The increase became greater as time went on p to death. An increase in the urea nitrogen is chiefly responsible for the ugmentation of the non-protein N, but the "undetermined fraction" also icreases. There is no significant change in the uric acid and the amino-cid N, sometimes a relatively small increase in the creatinine. The icrease in non-protein and urea N often precedes the development of erious symptoms. A concentration of the blood due essentially to an in-rease in the number and relative volume of erythrocytes can be detected i most cases somewhat in advance of the onset of symptoms. The change ien goes on increasing till death. It was stated that in a few instances i which the serum calcium was determined it seemed to be somewhat icreased with the onset of the symptoms. In table 1 of the present paper ie results of cholesterol and calcium determinations in 12 dogs (choles-rol in the blood, calcium in the serum) are given. These were additional ntrol dogs. They are mentioned in paper V of the series (table 2) Rogoff and Stewart, 1928), but the chemical work was reserved for the resent paper. Cholesterol was estimated by the Liebermann-Burchard action, always making duplicate determinations. Chloroform extrac-on was made in a Lieboff apparatus sometimes with the introduction of a ight modification. This consisted of the use of a plaster paris disc upon hich the blood sample (1 cc.) was distributed and dried. The disc rested on the absorbent paper disc usually employed in the extraction tube. number of determinations carried out in this manner, with known quan-

tities of added cholesterol, yielded very satisfactory results. To avo
evaporation of water from the bath, also condensation moisture on t
stoppers of the extraction tubes, a layer of paraffin oil was allowed to cov

TABLE 1

Cholesterol in blood and calcium in serum of adrenalectomised male dogs

RECORD NUMBER	DATE	CHOLESTEROL IN 100 CC. BLOOD	CALCIUM IN 100 CC. SERUM	RECORD NUMBER	DATE	CHOLESTEROL IN 100 CC. BLOOD	CALCIU IN 100 C SERUM
		mgm.	*mgm.*			*mgm.*	*mgm.*
128-9	4-25	192	13.0	130-4	5-18	170	11.0
	4-28	185	14.0		5-22	140	12.0
	4-30	150	15.5		5-23	152	13.0
	5-1	183	16.2		5-24	154	12.8
	5-2	136	17.0	130-8	5-11	185	11.0
	5-4	171	19.5		5-21	160	11.2
129-8	4-23	133	12.4		6-3	92	11.0
	4-25	132	13.3		6-4	120	10.8
	4-28	86	12.5		6-5	154	11.0
	4-30	120	12.0	130-9	5-6	196	11.5
	5-1	80	11.5		5-25	177	11.2
	5-2	150	12.5		6-3	204	11.4
129-9	5-11	142	11.5		6-5	240	12.5
	5-13	160	11.5		6-8	242	14.6
	5-15	160	11.5		6-10	208	15.0
	5-17	133	11.5		6-11	240	15.0
	5-20	85	12.8		6-13	172	15.8
	5-21	115	12.5		6-15	196	17.2
130-0	4-26	212	11.2	131-0	5-9	195	11.5
	4-29	184	11.0		5-25	178	11.4
	5-1	166	12.5		6-3	184	12.1
	5-2	160	13.0		6-5	202	13.0
130-1	5-18	171	11.0		6-8	218	15.0
	5-22	160	10.5		6-10	218	16.0
	5-23	172	11.0		6-11	236	15.6
	5-24	150	11.0	131-3	5-17	150	11.0
	5-27	170	12.5		6-1	172	11.4
130-2	5-18	196	12.8		6-6	215	11.5
	5-22	175	12.5		6-8	185	12.6
	5-24	171	12.4		6-10	162	14.5
	5-27	175	14.0		6-13	170	15.4
	5-28	194	14.8		6-15	133	17.0
130-3	5-4	218	12.0				
	5-9	245	13.2				
	5-11	266	15.0				
	5-13		16.5				

the bath. Calcium was estimated by Kramer and Tisdall's method
modified by Clark and Collip (1925). The data relating the events in t
history of the animals to the time of collection of the various blood spe

ıens are to be found in the condensed protocols following table 1. The drenal operations were done in the forenoon. Unless otherwise stated the lood was taken at 8:00 to 9:00 a.m. The last meal was on the previous ay about noon. In every case the bodyweight was well maintained ıroughout.

Dog 128-9. March 15, 1927 left adrenal and April 21 right adrenal removed. Quarıtine (snuffles) March 21 to April 5. Excellent health till April 28, when hallucinaons and convulsions developed. April 30, slight yelling fit. May 1, slight wobble ı walking. Eating less (biscuit and some meat); refused meal of meat. May 2, etter; ate fair portion of meat. Slight wobble in walking. Blood (dark) at 10:00 m. In evening the animal became apathetic. May 3, ate nothing, but is fairly ctive. Slight wobble. May 4, apathetic. Blood (dark) at 9:00 a.m.; slow flow. ay 5. Unchanged. Total anorexia. Not asthenic, although slight wobble. ay 6. At 10:30 a.m., unchanged. 3:00 p.m. to 11:00 p.m., worse. Some emesis oile); asthenic. May 7, dead at 6:15 a.m.; still warm.

Dog 129-8. On March 23, 1927 right adrenal and on April 21 left adrenal removed. xcellent recovery. Body-weight on March 28, April 4, 11 and 25, 9.2, 9.3, 10.25 and .6 kgm., respectively. Health was very good till April 29 and 30, when barking ells developed, but appetite remained good. Short convulsion on April 30. May quiet; ate little. May 2, convulsion (repeated 2 or 3 times) with coma. Total ıorexia. Some emesis. Blood obtained at 10:00 a.m. just after a convulsion. ay 3, emesis (bile) just before death at 8:45 a.m.

Dog 129-9. On March 23, 1927 right adrenal, on May 12 left adrenal removed. quarantine with cough 2 weeks before second operation. Torticollis developed oout 3 days after first operation and persisted till death. Health remained excelnt till May 19; appetite good; occasional emesis. On May 19, ate little, but fairly tive. May 20, decidedly less alert. Ate nothing. Not asthenic but slight wobble walking. May 21, semi-comatose; very asthenic. Blood (dark) obtained at 8:00 m., very slow flow. Died at 9:45 a.m. Weights on March 23, 28, April 4, 11, 21, , May 10, 12 and 17, were 7.0, 6.8, 6.8, 6.6, 7.25, 7.3, 7.5, 7.5 and 7.45 kgm., spectively.

Dog 130-0. On March 25, 1927 left adrenal, and on April 26 right adrenal removed. ood taken before second operation. Ate well till May 1; some emesis. April 29, short yelling spell in the morning. At noon ate good meal (meat), and had another lling spell. Later became quiet. Blood was taken at 10:00 a.m. On May 1 and l the end refused all food. At 8:30 a.m., good flow of blood during collection of ecimen. May 2, apathy; asthenia; anorexia; emesis (bile). Semi-coma. Died ıours after the blood specimen was taken. Blood dark; slow flow. Body-weight ll maintained.

Dog 130-1. On March 25, 1927 left adrenal and on May 19 right adrenal removed. cellent recovery. Is a moderate eater and apparently has recovered usual appee. May 22 to 25, unchanged. Ate fair quantities of meat but slowly. May 26, s alert. Ate very little (if anything). May 27. Refused all food. Somewhat athetic. Not at all asthenic. Tarry stools. Blood dark; slow flow. May 28. matose. Died at 9:00 a.m. Weights on March 25, 28, April 4, 11, 21, 25, 30, May 16, 19, and 22 were 7.05, 8.1, 8.1, 8.7, 8.9, 9.0, 9.6, 9.65, 9.6, 9.6 and 9.3 kgm., pectively.

Dog 130-2. Young animal. On March 25, 1927 left adrenal, and on May 19 right renal removed. Very good recovery. Till May 26, ate voraciously; was lively and gnacious. May 26, getting less active. Ate little. May 27, decidedly less active;

refused all food. May 28, apathetic, quite wobbly. Blood (dark) specimen 9:(a.m.; blood flow slow. At 4:00 p.m., emesis (bile). Apathetic and asthenic. Ma 29, dead in the morning. Weights on March 25, 28, April 4, 11, 21, 25, 30, May 1 16, 19 and 23 were 6.9, 6.55, 6.7, 7.3, 7.85, 8.0, 8.3, 8.4, 8.5, 8.7 and 8.5 respectivel

Dog 130-3. On April 19, 1927 right adrenal, on May 5 left adrenal removed. good health till May 11. Then began to get less active and to eat less. On May refused food; emesis. May 13 beginning asthenia; apathy; emesis (bile). At 10: p.m., comatose. May 14, in the morning was dead. Weights on April 19, 25, 3 May 5 and 10 were 7.2, 7.3, 7.75, 7.9 and 7.75 respectively.

Dog 130-4. On April 19, 1927 right adrenal, on May 19, left adrenal remove May 22, good condition, eating fairly well. May 23. Total anorexia; less activ May 24, apathetic but not asthenic. May 25. More apathetic. Eating nothin 6:00 p.m., stupor. At 9:00 p.m., dead. Body-weight had been maintaine throughout.

Dog 130-8. Left adrenal removed on May 12, 1927; right on May 31. Ate well June 1 (biscuit). On June 2 ate biscuit readily. June 3 refused food. Blood spec men (dark); slow flow. June 4. Stitch abscess (drained); short barking fi Took some biscuit but later refused it. Slow flow in getting blood specime June 5, apathetic; total anorexia; slow blood flow. June 6, unchanged. June dead in morning. Abscess around some stitches, not penetrating peritoneur Large ulcer in duodenum, not perforated.

Dog 130-9. Right adrenal removed on May 10, 1927; left on May 31. Conditic very good up to June 13, when he began to refuse food. Ate very little meat. Bloc sp. gr. 1,038 on June 10, 1,038 on June 13. June 14, somewhat less alert; refused a food; some emesis (bile). Head shakes slightly. June 15, slight wobble in walkin Total anorexia. Blood sp. gr. 1,042. June 16, dead at 7:30 a.m. Weights May 10, 31, June 7 and 13 were 11.35, 12.1, 11.9 and 11.4 kgm. respectively.

Dog 131-0. Young dog. Right adrenal removed on May 10, 1927, left on May (2:00 p.m.). Health very good till June 4, when he had a barking spell about noo (lasting 5 minutes). June 5 to 7, no more barking spells; appetite excellent, but June 7 some matter in eyes which condition as usual persisted till the end. June active; good appetite. Hair coming out (a common symptom at this stage). June Unchanged. June 10. Total anorexia, which continued till the end. Emesis (bile Short yelling fit (about noon, four hours after blood was drawn). Thereafter becan quite playful again, but took no food. Sp. gr. of blood 1,040. Slight wobble in wal ing in afternoon. June 11, quite asthenic; very apathetic; considerable wobblin At noon, semi-comatose. Died between 1:30 and 2:00 p.m. Weights on May 10, 2 31 and June 7 were 9.85, 9.95, 10.35 and 10.0 kgm. respectively.

Dog 131-3. Left adrenal removed on May 17, 1927, right on June 2. Good healt and appetite till June 14, although from June 10 on he is eating less than previousl Sp. gr. of blood on June 10, 1,046. On June 13, took a fair meal (meat); is very live and in excellent condition. Sp. gr. of blood 1,045. June 14. Little change. A biscuit, but not as greedily as before; refused meat; some emesis. June 15, sligl wobble; apathy; total anorexia; emesis (bile). This was in forenoon. In afternoo more emesis (bile); tarry stool. Blood sp. gr. 1,046. June 16, dead in mornin Weights on May 17, 23, 31, June 2, 7 and 13 were 12.95, 12.6, 12.7, 13.0, 12.75 ar 12.15 kgm. respectively.

Of the twelve dogs in table 1 more than half showed a decided increas in the calcium commencing with the onset of the serious symptoms occasionally somewhat preceding them (e.g., dogs 130-9 and 131-3). I

ıe dogs which did not show such distinct increases in the calcium as the ıajority there was nevertheless an almost constant tendency for the cal- ium to creep up. In two or three of the animals there was no change, even hen blood specimens were obtained at a time when marked symptoms ere present. Thus in dog 129-8, the last specimen was drawn just after convulsion and less than 24 hours before death, but the calcium was not ltered. In dog 130-8 also there was no change in the calcium content of ıe blood serum throughout the period of observation, and it was noted ıat in collecting more than one of the specimens the blood flow was slow ıd the blood dark. These specimens gave the same calcium content as ıose collected with a normal flow. It must be remembered that the blood ›ecimens were generally drawn at times predetermined by the routine and was not practical to make the intervals between successive specimens ›ry short. It is possible that sometimes a specimen taken nearer the end ight have shown a positive result although the last one was negative.

Our early results, already alluded to although not included in table 1, e practically the same as the later ones.

Thus, in dog 102-5 the right adrenal was removed on April 30, 1925 and the left on ne 5, 1925. The calcium on June 4 was 12.5 mgm.; on June 8, 11.6; on June 11, 14.3; June 12, 15.2; and on June 14, 17 mgm.

In dog 103-7 the left adrenal was removed on June 3, 1925 and the right on June 12. ıe calcium was 11.0 mgm. on June 11 and 14 mgm. on June 15.

In dog 103-8 the left adrenal was excised on June 3, 1925, the right on June 10 (in ›ernoon). The calcium on June 10 was 11.2 mgm. (Blood drawn in forenoon.) ı June 13 the calcium was 11.5 mgm.; on June 16, 12.5 mgm., and on June 18, 15.7 gm. Conductivity measurements towards the end (on June 16) showed concen- ıtion of the blood. This was also the case in dog 103-7.

Determinations of calcium were made on two pregnant dogs.

In 103-6 the left adrenal was removed on June 3, 1925; the right on June 12. The cium on June 11 was 11.5 mgm., on June 15, 13.4 mgm.; on June 19, 14.3 mgm.; June 23, 16.5 mgm.; on June 26, 13 mgm.; on July 9, 15.7 mgm. On July 28, in the enoon, the animal was comatose and received an injection of Ringer's solution at 00 a.m. Parturition began at end of injection but she was unable to care for the ee pups that were delivered during the afternoon. Died at 5:30 p.m.

In dog 103-4 the left adrenal was removed on June 1, 1925, and the right on June 9. June 8 the calcium was 10.7 mgm.; on June 13, 12 mgm.; on June 19, 12.5 mgm.; June 23, 12.5 mgm.; on June 26, 11.6 mgm. On July 3 a litter of pups was de- ›ered. On July 6 the calcium was 14 mgm; died today.

Data on the conductivity of the blood and serum, the concentration of › blood, blood counts, etc., are given in previous papers (Stewart and goff, 1925; Stewart, 1926), on dogs 102-5, 103-4, 103-6, 103-7 and other gs.

Discussion. We are not in a position at present to suggest any explana- n of the relation between the hypercalcemia, if it may be so denomi-

nated, and the metabolic or other changes associated with adrenal insu ciency. It is not due merely to concentration of the serum, for it is blood which is concentrated, not the serum. The blood has a much hig specific gravity and a much lower conductivity than before the co quences of the second adrenalectomy have developed. The specific gra and conductivity of the serum remain unaltered within the normal lir of variation. The fact is illustrated in some of the protocols (e.g. dog 12 that blood specimens collected immediately after the development very striking symptoms such as convulsions, or when the circulatio greatly slowed, may show no significant variation in the calcium cont so long as the animal continues to eat and the disturbance is transi This might be regarded as an indication that the increase in calcium s in the majority of the animals commencing simultaneously with or so what in advance of the onset of the characteristic symptoms of adre insufficiency is no superficial phenomenon but like the increase in n protein nitrogen, etc., a part of the picture of deranged metabolism duced sooner or later by loss of the adrenals. As to the relations betw known chemical changes and symptoms, any discussion would be p speculation at present.

As to the cholesterol, we do not think that any constant change rela to the clinical condition of the animals or the onset of characteristic sy toms of adrenal insufficiency can be demonstrated from the results in tab If in some of the experiments there may seem to be a diminution in cholesterol on the later dates, in others, and perhaps in the best and m complete series of observations (e.g., dog 130-9) this is not the case. conclusion were to be risked it might be that in fully half the cases cholesterol tends to diminish rather than to increase as the interval si the second adrenalectomy increases. Yet it would not be possible to tr such an inverse relationship between the cholesterol and calcium conte in some of the most complete experiments (130-9 and others). therefore, prefer to draw no conclusion from the cholesterol determi tions in table 1. They do not support the view brought forward by c tain writers, often based on histological observations only, that the a nals are peculiarly related to cholesterol metabolism. Results obtai by some observers on animals which only survived removal of the adre for a few hours or even a day or two, because of the inadequacy of surgical technique, cannot be considered as reflecting changes associa with adrenal insufficiency.

SUMMARY

The calcium content of the blood serum of adrenalectomised dogs generally found increased at the time of or sometimes a little preceding development of the serious symptoms, especially anorexia, which ter nate the period of good health.

The results on the cholesterol content of the blood do not permit us to onclude that any decided change in either direction is present either durng the period of good health or after development of the terminal ymptoms.

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STUDIES ON ADRENAL INSUFFICIENCY

VIII. The Survival Period of Untreated Adrenalectomised Ca

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Removal of the adrenals in the cat is easier than in any of the otl common laboratory animals except the rat. This has not prevented gr discrepancies in the results of different investigators as regards the time survival. Not only the older work but much of the newer presents a m of conflicting data, which cannot be employed as a standard, for examp in studying the effects of treatment upon adrenal insufficiency. In a case, we believe that an investigator, in order to be sure of his contr must accumulate them for himself. We have accordingly determined t time of survival and, as far as possible, the period of good health af removal of the second adrenal in about 50 cats, all healthy at the time the second operation. The results on 46 cats are given in tables 1 and If for special reasons a few cats have been omitted from the tables, th are mentioned in the text, and any reader who thinks they should go i the tables is free to put them in. It will not make any apprecia difference.

Twenty-eight cats in tables 1 and 2 lived 9 days or longer, i.e., m than four-sevenths of the total number. Between a fourth and a th of the animals lived 11 days or longer. A fourth of the 46 lived 13 d or longer. More than one-fifth survived 2 weeks or longer. One-eighth the animals lived about 20 days or longer. One cat survived $29\frac{3}{4}$ da one lived $31\frac{1}{2}$ days. The castrated cat which lived over 35 days will mentioned later. Cats therefore survive longer than dogs after bei deprived of the adrenals. The maximum period is considerably grea than that found by us in dogs. It is likely that with a greater number cats the progression from the shortest to the longest survival periods wou be more uniform, the gaps being better filled in, as in the control do Also, of course, the spread between minimum and maximum periods mig be increased.

As it is, there are no really wide gaps in the cat series nor any res which seems out of line with the rest. When an observer is in dou

TABLE 1

Adrenalectomised male cats

ECORD NUMBER	WEIGHT First operation	WEIGHT Second operation	TIME BETWEEN OPERATIONS	SURVIVED		ADRENAL WEIGHTS Left	ADRENAL WEIGHTS Right	PANCREAS CONGESTION	REMARKS
	kgm.	*kgm.*	*days*	*days*	*hours*	*grams*	*grams*		
12-2	3.12	3.5	15	$6\frac{3}{4}$			0.17	+	Old cat. Lower ileum ++
12-5	3.72	3.5	15	9			0.22	+	Alimentary canal +; 2 gastric ulcers
14-3	2.85	3.06	19	$19\frac{3}{4}$				+++	Stomach +, contains blood
14-8	2.4	2.2	19	$29\frac{3}{4}$				+	Weight 17th day, 2.12 kgm.
4-9	3.23	3.11	22	$8\frac{1}{2}$				++	Old cat
6-7	3.99	3.74	12	$6\frac{1}{2}$		0.21	0.26	+	Kidneys congested
6-9	2.95	2.72	12	10	4	0.12	0.15	+−	
7-0	2.72	2.65	12	10	21	0.20	0.22	+−	Bloody feces in rectum
7-3	4.11	3.69	10	14	3	0.25	0.29	++	Old cat; small intestine +; 2 small ulcers in pylorus
7-6	3.61	4.02	22	$13\frac{1}{2}$		0.23	0.25	−	
3-1	3.13	3.23	20	$19\frac{3}{4}$		0.14	0.18	++	Weight 12th day, 2.92 kgm.; 19th day, 2.75 kgm.
-2	3.11	3.22	20	$6\frac{1}{2}$		0.21	0.22	+	Stomach +. Emesis (bile) 5th day
-3	3.04	2.15	33	21	1	0.17	0.16	+	Lower bowel +; ulcer at pylorus. Weights 2.16 kgm. 16th day; 2.0 kgm. (20th)
1-4	2.02	2.53	32	$9\frac{3}{4}$		0.17	0.21	++	Stomach +; duodenum +. Weight 16th day, 2.37 kgm.
1-8	2.38	2.47	32	$6\frac{3}{4}$		0.21	0.18	++	Stomach contents bloody; two ulcers. Duodenum ++++; lower bowel + (blood)
1 3	3.23	2.74	24	$6\frac{3}{4}$		0.23	0.20	+++	
1 6	2.65	3.1	25	$9\frac{3}{4}$		0.21	0.21	++	Rectum +
1 7	3.12	2.91	25	5	$1\frac{1}{2}$	0.24	0.22	+	
1 8	2.73	3.3	23	$9\frac{3}{4}$		0.25	0.18	+	
1 9	2.13	2.15	23	$10\frac{3}{4}$		0.17	0.18	+++	Small intestine ++; large intestine +
15 0	3.39	3.37	23	10	5	0.22	0.18	++	Stomach and intestines +; 2 erosions near pylorus. Accessory removed at 2nd operation

TABLE 1—*Concluded*

RECORD NUMBER	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED		ADRENAL WEIGHTS		PANCREAS CONGESTION	REMARKS
	First operation	Second operation				Left	Right		
	kgm.	*kgm.*	*days*	*days*	*hours*	*grams*	*grams*		
157-1	2.35	2.4	23	$5\frac{1}{2}$		0.31	0.22	+	
157-3	3.39	3.57	23	$7\frac{3}{4}$		0.36	0.24	+++	Old cat. Small intestine +
157-4	3.4	3.57	23	9	5	0.27	0.26	+++	Erosion near pylorus; duodenum +−
157-5	2.42	2.57	24	10	2	0.24	0.20	++	Duodenum ++
158-3	3.26	3.23	17	$14\frac{3}{4}$		0.22	0.17	+	Ulcers in pylorus; large intestine ++

In cats 146-7 to 148-8 inclusive the left adrenal was removed first, in all the others last. The + signs in the remarks refer to congestion of the gastro-intestinal tract.

whether one or more of his results are really out of line in a small series, the test is to see if with a larger series the gaps tend to be filled in. None of the excluded animals lived as short a time as the minimum in the tables. All the cats operated on recovered from the second operation. None died in as short a period as 1 to 2 days.

The average survival period for the 26 male cats in table 1 is 11 days, and for the 20 non-pregnant female cats in table 2, it is $10\frac{3}{4}$ days. As noted in connection with the dogs, we do not think the averages of quantities which vary as much as these periods of survival are of much value especially in small groups of experiments. Certainly the conclusion should never be drawn that because the average duration of survival with a given treatment is somewhat greater than in the controls, the treatment must have been efficacious in "substituting" for the adrenal cortex. It is essential that a fair proportion of the treated animals should survive well beyond the maximum of the controls. As control cats live longer than control dogs, treated cats must live longer than treated dogs in order that a positive result may be inferred.

It was not thought worth while to watch the cats through the night as was done with most of the dogs. When a cat, which had been alive the previous evening, was found dead in the morning the extra fraction of a day was taken as $\frac{3}{4}$. The operations were done from 9 to 10 a.m., so that the animal would have completed half a day when seen late in the evening before death. It is believed that while some of the cats may have lived a few hours less than $\frac{3}{4}$ day, others would live a few hours longer and in a large series of animals the result would come out about even. When the animals were seen dying the exact survival time is, of course, given.

TABLE 2

Adrenalectomised non-pregnant female cats

RECORD NUMBER	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED		ADRENAL WEIGHTS		PANCREAS CONGESTION	REMARKS
	First operation	Second operation				Left	Right		
	kgm.	*kgm.*	*days*	*days*	*hours*	*grams*	*grams*		
142-3	2.62	2.75	15	$9\frac{3}{4}$				+	
142-4	2.81	2.68	15	$6\frac{1}{2}$			0.13	+	
142-6	4.17	3.9	14	$8\frac{1}{2}$			0.28	−	Pancreas mottled, white spots, no congestion
142-8	2.53	2.63	15	$7\frac{1}{2}$			0.21	+−	Two ulcers in pyloric end of stomach; duodenum +
142-9	2.7	2.68	15	8	3		0.15	+	
144-0	2.48	2.28	23	$7\frac{3}{4}$				+	
144-1	3.23	3.1	46	$31\frac{1}{2}$				++	Had 1 kitten a month before 2nd operation
148-5	1.97	2.01	32	$7\frac{3}{4}$		0.15	0.13	+−	Young cat. Three ulcers (pyloric end). One in duodenum
148-6	3.87	3.25	32	$14\frac{3}{4}$		0.22	0.23	++	Ulcer in pyloric end and 1 in duodenum. Weight 16th day, 2.98 kgm., 14th day, 2.89 kgm.
148-9	1.95	1.65	32	$13\frac{1}{2}$		0.22	0.12	++	Weight (6th day) 1.36 kgm., 13th day, 1.35 kgm.
152-4	1.92	1.8	9	$2\frac{3}{4}$		0.15	0.16	++	Youngest cat
152-6	3.89	3.78	9	$13\frac{3}{4}$		0.26	0.32	++	Ulcer near pylorus. Large bowel ++++ and bloody contents
152-7	2.73	2.66	9	$9\frac{3}{4}$		0.24	0.19	++	Parturition 5 weeks before 1st operation
152-8	1.72	1.83	25	9		0.23	0.17	++	
152-9	3.11	3.06	25	$9\frac{3}{4}$		0.17	0.18	+−	Young adult. Gastric ulcer; small intestine +
153-0	2.33	2.46	25	8	$5\frac{1}{2}$	0.20	0.18	++	Stomach two ulcers. Old cat
153-2	1.72	2.08	25	$9\frac{3}{4}$		0.27	0.26	++	An accessory removed at 1st operation
158-1	2.43	2.5	17	$11\frac{3}{4}$		0.27	0.27	++	Ulcers in stomach. Duodenum +
158-4	2.4	2.57	17	$5\frac{3}{4}$		0.20	0.17	+++	Had litter 2 months before 1st operation
158-5	2.13	2.29	17	$19\frac{3}{4}$		0.22	0.18	++	Had litter 6 weeks before 1st operation

In cats 148-5 to 148-9 inclusive and in 152-8 to 153-2 inclusive the left adrenal was first excised, in the others last.

As in dogs, the period of good health is usually 2 to 3 days shorter tha the survival period and does not vary with the duration of survival. Bı the onset of the terminal symptoms, especially the anorexia, is often n as easy to determine as in dogs owing to the difference in the habits ar psychology of the two animals. It is more common among cats tha among dogs to find animals which continue to eat up to about a day befo

TABLE 3

Adrenalectomised pregnant cats

RECORD NUMBER	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED		ADRENAL WEIGHTS		PANCREAS CONGESTION	REMARKS
	First operation	Second operation				Left	Right		
	kgm.	*kgm.*	*days*	*days*	*hours*	*grams*	*grams*		
147-5	3.2	2.94	10	18¾		0.27	0.25	+−	Advanced pregnancy 2nd operation; 3 kitte day after operation
148-0	1.84	1.9	20	5		0.14	0.15	++	Early pregnancy. Stoı ach, 3 ulcers near p lorus
152-5	3.6	3.43	9	2½		0.22	0.18	−	Advanced pregnancy. Coma and in labor days after 2nd oper tion
153-1	2.89	2.92	25	8	4	0.18	0.21	++	About term. Stomach, ulcers
153-5	2.35	2.53	7	4½		0.22	0.25	++	Advanced pregnancy embryos). Stomach. large ulcers
155-9	2.65	3.05	19	8¾		0.17	0.15	++	Four fetuses (about mm.) born 4th day aft 2nd operation. Ulc in stomach
156-0	2.6	2.46	19	9	4	0.22	0.25	++	One fetus born 2nd da after 2nd operation; ı more

Left adrenal removed first in cats 147–5, 148–0 and 153–1. In the others it w removed last.

death. For this reason it is more common to find at autopsy food in tl stomach of cats, although we have occasionally seen this in dogs, and dog has been seen to eat a full meal the day preceding death. Somethir depends upon the nature of the food.

In cat 147-2, a male, the left kidney was about 3 times as large as the right whi was normal. The capsule of the left kidney was maculated, thick and hard. C

ection, intense stasis affecting equally cortex and medulla was revealed. On the ower pole of the kidney under the capsule was a blood clot with signs of recent emorrhage. It is not known to what extent this condition was a factor in the elatively short survival ($4\frac{3}{4}$ days). For this reason the cat is not placed in table 1. There was no congestion of the pancreas or gastro-intestinal tract.

Cat 147-9, a male, lived $3\frac{3}{4}$ days. At autopsy blood was found in the pericardium. The origin of this blood is not known, but it was thought best not to include the at in table 1.

In cat 155-7, a female, at the operation for removal of the first adrenal (right) two mall accessory nodules (about 1 and 2 mm. in diameter, respectively) were seen t the site of the gland, with the usual vessels and nerves around them. No adrenal ould be found. The accessories could have been taken away but it was desired to ee whether they would sustain life. After 15 days the left adrenal was removed. The cat lived 14 days, eating little or nothing from the 7th or 8th day onwards. At utopsy the two accessories were found and were of the same size as before. A mall vein came from each. They were shown microscopically to consist of cortical issue. A fibrosed mass about the size of a normal adrenal was found where the ight adrenal should have been. It was attached by the lumbo-adrenal vein to the ava. Another small accessory (identified microscopically) was found on the cava ust beneath the liver. The pancreas was considerably congested (++). There vas some congestion of the jejunum in scattered patches. The ileum showed noderate hemorrhagic congestion. The large intestine was somewhat congested. The right ovary was almost completely destroyed by cystic degeneration. Because f the presence of accessories, not, however, sufficient to save, or perhaps even to rolong the animal's life, this cat is not included in table 2.

In two of the cats (157-0 and 153-2) included in tables 1 and 2, a small accessory as seen on the adrenal vein on the left side and removed at the operation. No ccessories were found *post mortem* in any of the cats although most careful search as made in every case.

Two cats (147-4 and 153-4) had been castrated, probably long before they were rought to the laboratory. Opinion is not unanimous as to the effect, if any, of astration upon the survival period after adrenalectomy. So far as we can see om an examination of the literature, there is no reason to conclude that there is ny definite effect in either direction. But it was considered best to exclude them om table 1. One of the cats (153-4) lived a shorter time ($7\frac{1}{2}$ days) than the majority the control animals. It was an old animal. It weighed about 4 kgm. At autopsy abscess was found in the left lung and the right lung was infiltrated. How much, at all, this contributed to shorten life is unknown. The other cat (147-4) weighed er 4 kgm. It was very fat. It survived longer than any other control cat we have erated upon (35 days 3 hours). Both are excluded from table 1.

As regards the operation and the care of the animals before and after everything said in paper I (Rogoff and Stewart, 1926) on the dog holds ue. It is even more important to have well heated quarters, for the cat, small animal, is more easily injured by cold than the dog. This is of urse particularly true of cats soon after the operations. We have seen ts die after excision of one adrenal, undoubtedly from this cause, and we ve never seen that in dogs. A cat had one adrenal removed. Its condion after the operation was excellent. Two days later, heat was turned in the building except the rooms in our laboratory which have a separate

supply of steam. It so happened that the room in which the cat wa received no heat for more than 24 hours; the weather turned suddenl cold and the next morning the cat was dead. It is scarcely necessary t prove that cold quarters will harmfully affect a small animal recentl operated on.

Shortening of the operation and the anesthesia is quite as important a in the dog. As already stated, adrenalectomy is a very easy operatio cats. The usual time from the skin incision to closing of the wound with us about 5 minutes.

Symptomatology. In our published papers (Rogoff and Stewart, 192 1926, 1927, 1928) on this problem which, although a single continuou investigation, is reported for convenience in separate installments, we gav a detailed account of the symptoms in dogs, since the animals studied b previous observers died too soon to permit accurate observations. Fo cats the symptoms are well described by Elliott (1914). Between cats an dogs certain differences exist, e.g., emesis is less common in the cats. definite onset of anorexia is often less easy to establish. We have there fore not put into our tables the length of the period of good health. Con vulsions and hallucinations are less common than in dogs but are seen Blood in the stools is more rare than in dogs.

Post-mortem appearance. The striking congestion and often hemorrhage in the gastro-intestinal mucosa with blood in the lumen which were note in the dogs are less common in cats. When present, congestion is usuall less intense and less widespread, while blood in the lumen is occasionall found. Feces in the rectum are sometimes blood-stained. Ulcers i the stomach are more common than with dogs. As in the dog, bile is ver frequently found in the stomach. The pancreas is nearly always mor or less, not seldom, markedly congested. If the congestion of the pancrea appears less intense in many cases, this may be because of the much smalle thickness of the congested tissue, also of course the smaller size of the veins There is no doubt, however, that qualitatively at least the condition similar to that in the dog. It may be mentioned that this is also the cas in the guinea pig. The presence of this condition in animals dying of adre nal insufficiency may not only afford another indication of an associatio between the pancreas and the interrenal tissue, but may be among th contributing causes of death.

Tokumitsu (1923) has suggested the existence of a correlation between the corte and the pancreas, especially in relation to the metabolism of carbohydrates. How ever, it is impossible to see how he could have arrived at any conclusion because o the extremely poor operative results, rats dying 4 to 20 hours and rabbits 10 to 2 hours, after removal of the adrenals.

The loss of appetite for fats and interference with their digestion in Ad dison's disease is suggestive of a correlation between the adrenal corte

nd the pancreas. The aversion to fat is a prominent symptom, appear-ıg as part of, or preceding, the onset of anorexia in adrenalectomised dogs. drenal insufficiency is of course more acute in these animals than in uman cases where some interrenal tissue is still functioning. Under eatment with extracts the aversion to fats has been seen to become less ıarked or to disappear in dogs. Amelioration of the symptom (along ith other symptoms) in cases of Addison's disease treated during the past vo years with extracts (interrenalin) was observed.

It would be a waste of space to quote a great part of the literature as it is without lue. Moore and Purinton (1900) state that out of 15 cats 2 died after removal of e first adrenal, one of peritonitis, one of general septicemia, and one was killed by loroform because of a septic condition of the wound. Of the remaining cats from ıich the glands were removed in two operations, 7 survived as follows: three der 24 hours; one, 24 hours; one, 33 hours; one, 2 days and 4 hours; one, 4 days and hours. The results were thus extremely bad.

Gradinescu (1913) removed the adrenals in one stage from 9 cats. They survived , 16, 20, 30, 40, 55, 56, 84 and 96 hours respectively (average $45\frac{1}{2}$ hours). As dogs erated on in 2 stages survived only for an average period of 42 hours, and rabbits erated on in one stage only 7 hours, it is obvious that the technique was very poor.

The much quoted observations of Strehl and Weiss (1901) gave survival periods cats doubly adrenalectomised in one stage as follows: 15 cats, 15 to 28 hours; 2 s, 28 to 47 hours. Five cats from which the adrenals were removed in two stages th an interval of a month, lived 30 to 170 hours after the second adrenalectomy. ese are poor results, and anyone who trusts to them, or others as poor, as furnish-; a control series for testing the effect of treatment on cats is bound to come to oneous conclusions. The same may be said of the data of these observers on dogs l other animals. The survival periods are much too short; and the technique ınot have been good.

H. A. Stewart (1914), working on the question whether the adrenals are related to ɔlesterinemia, found that among the rats employed a pregnant animal was the gest survivor after removal of the adrenals. He recognized that rats were quite suitable for such experiments on account of the frequency of indefinite survival. then used cats and rabbits with a few guinea pigs to see whether pregnancy and tation exercised an influence on the survival period. The observations on rabbits rcely require mention because out of 10 animals there was only 1 case of in-inite survival (1 year). Lactation at the time of adrenalectomy could of course e had nothing to do with the long survival. Four other lactating or pregnant bits survived as follows: one, 1 day; two, 2 days; and one, 6 days. Of the control bits, two lived 1 day; one, 6 days; one died (on the table ?). These are extremely r results for rabbits.

)ne lactating guinea pig lived 1 day; two, 2 days; one, 5 days; one, 7 days; one l on the table. Only one control guinea pig is mentioned. It lived 3 days. 'iously no conclusion can be drawn from these results even if it was certain that whole of the adrenals, especially the right, was taken away. In one (lactating) ıea pig which is described as being alive more than 3 months after removal of second adrenal it is extremely likely that some of the cortex was left behind.

)f the pregnant and lactating cats, one lived half a day; two lived 1 day; five, 2 s; one, 3 days; one, 4 days; one, 6 days; three, 7 days; two, 9 days; two, 10 days; , 11 days. Two died on the table, and three were killed (reason not stated).

Of the control cats, one lived less than a day; twelve, 1 day; seven, 2 days; one, : days; eight, 3 days; three, 4 days; two, 5 days; one, 6 days; one, 7 days; one,] days. One cat died on the table and several were killed for various reasons.] some cases the experiments were complicated by other operations, and injectio of lanolin, etc. In both series many of the animals became infected.

These results are not good enough to permit any conclusion to be draw In the paper, however, an attempt is made, by eliminating many of tl animals for various reasons, to prove that lactation and pregnancy increa the average duration of life in cats. The symptoms also are stated to l "all much delayed and mitigated." We believe that comparing averag of such figures as are given in the two series is fraught with error, partic larly, when a more or less arbitrary selection of results is made.

In any case, the conclusion is not borne out by our own observatio (table 3). While in dogs we found in pregnant animals and animals : heat clear evidence of a protective influence, it was not so in pregnant cat It is true the number of pregnant cats was not great, only seven, but y perhaps sufficient. The results were certainly no better, perhaps somewh worse than in the controls. We made no observations on the influence heat in cats. We think it unlikely that there should be any essenti difference between dogs and cats in a matter of this kind. We may expe it to be more difficult to demonstrate an influence of pregnancy in ca than in dogs. Pregnancy as such is an additional, often a serious, hand cap to animals subjected to an adrenalectomy. Even if the protecti effect would eventually come into play, the animal may not chance to li long enough to get the benefit of this. We saw, not seldom, in dogs cas where the pregnant animal could not overcome the additional handica and the survival period instead of being lengthened was shortened. Pr mature labor can, undoubtedly, be brought on by the second adrenal oper tion (more readily in cats than in dogs) and it depends on the condition the animal, perhaps on the stage of pregnancy, whether it will successfull cope with the added strain or succumb. In spite of this difficulty, we we able to prove in dogs that the conditions under discussion could prote since many of the pregnant animals (and all of those in heat) lived de cidedly longer than any of the control dogs. As stated, we do not thin that the relatively poor results in the pregnant cat indicate that there i no potential protective influence of the pregnancy changes, but it wi be more difficult to demonstrate them. Corey (1927) was also unable t demonstrate a protective influence of pregnancy in cats. In his contr animals the onset of anorexia invariably occurs 50 to 58 hours after the re moval of the glands. He finds the average life span of untreated operate cats is only 100 to 120 hours (4 to 5 days). In our experience this is too low but his results are better than those of Zwemer (1927) who states that cat deprived of both adrenals will survive an average of 53 hours, the extreme being 26 and 111 hours (1 and $4\frac{1}{2}$ days).

The greater susceptibility of pregnant cats than of pregnant dogs to e operation itself is well illustrated by cat 157–2, which, of course, not included in table 3. The animal was in advanced pregnancy when e first (right) adrenal was removed. She died $4\frac{1}{2}$ days later with her 't adrenal still intact. Coma developed on the second day. Clearly, e cat did not die from adrenal insufficiency but probably from some mplication associated with the pregnancy and brought on by the opera- on. Observations on the effect of heat in cats on the survival period are sirable, as it is scarcely likely that heat would be in itself a serious ndicap.

Elliott (1914) found that of 25 cats from which the glands were removed in two ges, 9 died on the second or third day. Thirteen lived 6 to 10 days; two survived the 22nd and 23rd day and one was sacrificed in the 9th week. He attributes longer periods of survival to compensatory hypertrophy of small accessory renals. We presume that the last three cats should be omitted for our purpose. Marine and Baumann (1927) obtained the following survival periods in 18 cats: , 2.5, 2.8, 3.0, 3.5, 4.0, 4.2, 4.3, 5.0, 5.2, 5.2, 5.5, 5.8, 7.0, 7.0, 7.5, 8.8 and 12 days. cluding a kitten which lived 22.3 days, they take 5.3 days as the average period of vival. The kitten was excluded, though no accessory was found, because it s "so out of line with others of this series as well as with our experience of several ndred other suprarenalectomised cats."

A grave defect in the work of Hartman, et al. (1928) on "prolongation of by extracts" is that they have not published satisfactory controls of ir own. To select from the literature a series obtained by other workers l then to use them for comparison with their own treatment series is not, our opinion, a proper procedure, for more than one reason. Our results w that the survival period of normal cats is far greater than appears in y of the series hitherto published. As a matter of fact, out of more n 90 cats treated with extracts by Hartman and his collaborators only ved much beyond the maximum shown in our 46 control untreated s (tables 1 and 2), and 3 somewhat exceeded our maximum.

n another paper Hartman (1926) published what we should consider y poor results in a series of cats injected with material of various ls. As some of this material was almost certainly harmless the cor- ponding observations constitute a sort of control although not intended ich. Seven cats adrenalectomised at one sitting received intramuscular intravenous injections of adrenalin and survived on the average only 9 hours. Of the animals that received extracts of adrenal cortex of ious kinds 5 lived an average of 60 hours; two of 45 hours; 9 of 40 rs; one lived 93 hours; 22 (injected with saline extracts of cortex) an age of 146 hours (about 6 days). These results are not nearly as good lliott's on untreated cats and very much inferior to our control cats les 1 and 2). It is impossible to deduce from them any protective

influence of the extracts used. MacArthur, Dean and Hartman (19[illegible]) observed a group of 13 adrenalectomised cats some of them receiving injection of extracts. The group lived 5 to 26 days (average 12.7 days). This constitutes a negative result when compared with our control table

SUMMARY

The survival periods of 46 untreated, adrenalectomised cats, (control series) are tabulated. The maximum period of survival of the cats included in the tables is $31\frac{1}{2}$ days. The average survival period of 26 male cats (table 1) is 11 days; of 20 non-pregnant female cats (table 2) [illegible]$\frac{3}{4}$ days. This is very much longer than anything hitherto reported.

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STUDIES ON ADRENAL INSUFFICIENCY

IX. THE INFLUENCE OF EXTRACTS OF ADRENAL CORTEX (SHEEP AND CATTLE) ON THE SURVIVAL PERIOD OF ADRENALECTOMISED DOGS AND CATS

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In paper V of this series (Rogoff and Stewart, 1928) the influence of dogs' adrenal extracts in lengthening the period of survival in dogs after total adrenalectomy (in two stages) was demonstrated. A not inconsiderable number of the treated animals survived far beyond anything seen in untreated controls. The period of good health seems to be correspondingly prolonged, perhaps relatively even more than the total survival period. Not seldom the animal continues to eat almost up to the end, so that it may be impossible to notice the onset of anorexia as a definite event presaging death, as is usual in the controls some days before death. At present we do not deduce a positive conclusion from a modification of the symptomatology, although we think this may be observed, unless in a considerable number of the animals the survival period is increased without question beyond the limits seen in the controls. We have also gained the impression that the pathological changes in the gastro-intestinal mucosa are modified by treatment with extracts, hemorrhagic congestion in the mucosa being less common and less extensive. This may be the case even in animals which do not outlive controls. The congestion of the pancreas is not affected.

In table 1 are assembled the results of observations on 32 dogs treated by intravenous injections of extracts of cortex (interrenalin) from abattoir material, in this case entirely from sheep. The injections were made once daily, the dose varying from 1 to 2 cc. The injections were well borne, no ill effects being seen. Six dogs lived beyond the maximum period seen in the controls ($16\frac{1}{2}$ days, $17\frac{1}{3}$ days, $17\frac{3}{4}$ days, over 19 days, $20\frac{1}{2}$ days, [illegible]$\frac{1}{2}$ days). Four dogs lived practically for the maximal period of the controls ($14\frac{5}{6}$ days, 15 days, $15\frac{1}{4}$ days, $15\frac{1}{2}$ days). The ten unusually long survivors were in a series of but 32 dogs. A much smaller proportion of animals running beyond 14 days was seen in the controls (at least 200 in number)—only 2 in table 1, paper I (Rogoff and Stewart, 1926) in 29 male dogs; none in 39 non-pregnant female dogs in table 2, paper I; and 4 in 36 dogs in

ble 2, paper V (1928). Altogether, only 6 dogs in the 104 control dogs in ese three tables lived over 14 days and none reached 16 days. It is clear at the treated adrenalectomised dogs present us with a new picture,

TABLE 1

Adrenalectomised male dogs treated with extracts of sheep's adrenal cortex (intravenously)

RECORD NUMBER	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED		BEGAN TO REFUSE FOOD	ALIMENTARY CANAL		PANCREAS CONGESTION	REMARKS
	First operation	Second operation					Blood	Congestion		
	kgm.	*kgm.*	*days*	*days*	*hours*	*days*				
2-4	9.3	9.1	23	11¾		10	+−	0	++++	
2-5	10.9	10.4	23	12¼		12	+	+−	+++	
2-6	8.8	8.7	23	19	2	17½	+	+	++++	
3-6	10.8	10.6	24	10¾		9	+	+−	++	Small ulcers (pylorus and duodenum)
3-7	11.1	12.3	24	7½		5	+	+	+	Coughing at 2nd operation
3-8	8.2	8.5	24	15½		15	0	0	++++	
3-9	9.6	10.0	27	11¾		11	0	0	+++	
4-5	11.2	11.0	27	7¾		6½	++	++	+++	Small erosions (pylorus)
4-6	9.4	9.8	21	10¾		9	0	0	+++	
5-9	11.3	11.7	27	20½		19	+	+	++++	
6-0	7.5	8.2	27	7¾		5½	0	+	+++	
6-2	14.9	14.0	16	13¾		13	0	0	++	
6-3	10.8	10.8	16	10	20¼	9	++	+	++++	
6-4	13.3	13.5	16	8¾		7	0	0		
7-0	11.8	12.1	36	8	22	6	+++	+	++	7 small ulcers (pylorus); 1 larger ulcer (duodenum)
7-1	11.8	11.8	36	16½		14½	++	+	+	
7-4	19.9	19.4	30	22½		21	0	+−	+	Small erosions (pylorus)
7-5	15.3	13.5	41	13	22½	11	+−	+−	+++	Very mangy
7-6	15.9	16.3	41	15	0	15	0	0	+++	Erosions (pylorus)
9-8	9.0	9.6	35	9¾		8½	+	+	++++	
9-9	11.8	12.3	35	13	15	10	+	+	+++	Many small ulcers (pylorus)
0-0	11.1	11.0	31	17¾		15	0	0	+++	
0-2	10.6	11.3	30	10¾		10	+++	+++	++	
0-3	13.2	13.2	30	8½		7½	0	0	++++	
0-4	14.8	15.2	29	12	0	9	++	++	+++	
6-2	17.7	19.4	18	10	2	5	++	+++	++++	Ulcers (pylorus and duodenum)
6-3	18.2	17.0	18	14	20	13½	+−	+−	+++	
6-5	16.0	16.9	17	15	6	15	++	++	++++	
0-1	18.3	19.9	69	8¾		6	+++	+++	++++	
2-0	15.4	16.2	36	12	0	10	+++	+++	++++	Many ulcers (stomach)
2-1	16.0	15.5	36	17	8	15½	++++	++++	++++	
2-2	16.5	16.3	36	6	0	4	++++	++++	+++	

mething not seen in the controls in the relatively large number of animals rviving decidedly beyond the maximum observed in the controls. Long rvivals not surpassing the maximum are more common than among the

controls. If table 1 is analysed on the same basis as table 1, paper V (1928), which contains the results with intravenous injection of extract of dogs' adrenals into dogs, six animals are found to surpass the maximum in the controls, the total number of animals being practically the same for the two kinds of material. In addition, however, there are in table 1 of the present paper four animals which lived from $14\frac{5}{6}$ to $15\frac{1}{4}$ days, whereas there are none in this group in table 1 of paper V. That table contains 7 dogs

TABLE 2

Adrenalectomised male dogs treated with extracts of sheep's adrenal cortex (subcutaneously)

RECORD NUMBER	WEIGHT: First operation	WEIGHT: Second operation	TIME BETWEEN OPERATIONS	SURVIVED		BEGAN TO REFUSE FOOD	ALIMENTARY CANAL: Blood	ALIMENTARY CANAL: Congestion	PANCREAS CONGESTION	REMARKS
	kgm.	*kgm.*	*days*	*days*	*hours*	*days*				
132-3	9.8	9.2	23	$15\frac{2}{3}$		$14\frac{1}{2}$	0	+−	+	
134-1	11.2	11.0	27	$7\frac{2}{3}$		$6\frac{1}{2}$	++	++	+++	Small erosions (pylorus)
134-6	8.9	9.1	21	10	$22\frac{1}{2}$	10	0	0	+++	
137-2	9.9	8.4	37	$10\frac{1}{2}$		8	+−	+	++	
137-3	17.5	16.1	37	$13\frac{3}{4}$		12	0	0	++	Ulcers (pylorus and duodenum)
138-6	19.3	18.9	19	7	$21\frac{1}{2}$	6	0	+	+++	
138-7	14.4	14.3	33	$9\frac{1}{2}$		7	++	+	+++	Many small ulcers (pylorus); large ulcer (duodenum)
139-0	11.1	10.1	28	$8\frac{1}{2}$		8	0	+−	++	
139-1	15.4	14.8	28	9	12	9	0	+	+	Mangy
139-3	17.2	16.4	29	7	5	4	+++	++	+++	Goiter. Spleen enormously congested
141-0	14.4	13.4	20	10	18	9	0	+	++++	4 small ulcers (pylorus)
141-4	13.4	12.7	36	23	$3\frac{1}{2}$	21	++	++	+++	Old dog. Ulcers (pylorus)
141-5	15.4	15.0	38	6	3	3	++++	++++	+++	
141-7	9.9	8.1	37	$6\frac{3}{4}$		$5\frac{1}{2}$	0	+	++	
141-9	11.4	11.7	22	$14\frac{3}{4}$		14	0	+	+++	3 ulcers (pylorus)
143-4	19.6	19.2	28	$4\frac{3}{4}$		$2\frac{1}{2}$	+++	++	++	
143-5	13.4	12.8	28	7	$1\frac{1}{2}$	$6\frac{1}{2}$	+	+	++++	Erosions (pylorus)
143-6	12.5	12.4	28	$11\frac{1}{4}$		9	+	+−	+++	Small erosions (pylorus and duodenum)
143-8	9.3	9.1	28	$11\frac{3}{4}$		8	++++	++++	+++	

surviving from 11 days to $13\frac{1}{2}$ days, against 12 dogs living from practically 11 days to 14 days in table 1 of the present paper. It is clear that there is no essential difference between the results of intravenous injection of homologous and heterologous material. Nor should any difference be expected from what is known of active substances from other endocrine glands. That no animal in the present series approached the exceptional survival period of 78 days seen in one dog of the previous series may be considered accidental. It is not known what favorable circumstances

ıabled that animal to live so long. The important point is that it was certained that no remnant of adrenal tissue was left behind, nor did the ost careful and prolonged search reveal any accessory adrenal. The sults of our extensive control series leave no doubt that the long survival as due to administration of the extracts.

The period of good health is lengthened fully as much as the total survival period. Not seldom the animal continues to eat, at least some food, l within a short time before death (often 1 day or less). We do not trust uch to averages of survival periods for reasons given in previous papers, ıt the average is about 13 days for the treated animals in table 1.

TABLE 3

Adrenalectomised male dogs treated with extracts of sheep's adrenal cortex (by mouth in coated capsules)

RECORD NUMBER	WEIGHT: First operation	WEIGHT: Second operation	TIME BETWEEN OPERATIONS	SURVIVED		BEGAN TO REFUSE FOOD	ALIMENTARY CANAL: Blood	ALIMENTARY CANAL: Congestion	PANCREAS CONGESTION	REMARKS
	kgm.	*kgm.*	*days*	*days*	*hours*	*days*				
6–1	10.0	9.9	47	8¾		7½	+++	+++	+++	Several small ulcers (pylorus)
6–2	10.0	11.1	48	10¾		6	+++	+++	+++	
6–3	14.8	15.0	35	11	½	9	++++	++++	++++	Erosions and ulcer (pylorus)
6–4	14.0	13.6	46	8	20	7	+++	+++	++	
6–6	9.1	10.2	46	6¾		5	++++	++++	++	
9–0	11.6	11.1	27	11½		10½	+++	+++	++++	Ulcers (pylorus and duodenum)
9–1	10.0	9.8	27	9	5	7	++++	++++	+++	
9–4	13.6	13.2	23	10	19½	9½	++++	++++	+++	
9–5	12.7	13.3	24	9	21	7	++++	++++	++	
9–6	11.7	11.4	22	16	3	14½	+++	+++	++++	Capsules and dropper; ulcers (duodenum)
0–1	12.4	11.6	31	11	7½	10½	++	++	++++	Ulcers (pylorus)
6–4	17.3	17.6	16	13⅔		12	+++	+++	++++	Ulcers (stomach)

In table 2 are assembled the results of subcutaneous administration of rtical extracts (sheep). The table includes 19 dogs. The extracts were nilar to those injected intravenously. Only one dog (141-4), obviously ı old animal, survived beyond the maximum of the controls, living indeed 'er 23 days, or a week more than any control dog. Two other dogs lived yond 14 days (14¾ and 15⅔). Save for the dog which survived 23 days e result would be considered negative. The average survival period for e 19 dogs is 10.4 days. The average of the control dogs in table 2, paper , is 9.6 days. The result may perhaps be considered as slightly positive, ıt subcutaneous administration of our extracts in dogs appears to be uch inferior to intravenous administration. It is quite possible that,

judged by this very severe test of an increase above the maximum survival period (extension of survival beyond the maximum seen in the controls), subcutaneous injection may give a negative result in the great majority of the cases with an exceptional positive result. We have formed the impression that old animals usually bear the loss of the adrenals better, i.e., survive longer than young animals. This is not ascertained, it is, as said, an impression.

In table 3 are included a small series of 12 dogs treated orally with the same extracts as used for the animals in tables 1 and 2 except that the proteins were not precipitated and the dose was greater. In nearly all cases the extracts were given in coated capsules, expected to pass through the stomach without being destroyed. Sometimes the dogs were with difficulty induced to swallow the capsules. Some dogs would discover them in the food and carefully eliminate them. On the whole, however, there is no doubt that they all took the chief part, at least, of the doses given them. Occasionally recourse was had to pouring the extract into the back of the pharynx with a medicine dropper.

The results in table 3 are negative. Only one of the animals lived as long as 16 days. Two lived $13\frac{2}{3}$ days. Three lived 11 to $11\frac{1}{2}$ days; two about $10\frac{3}{4}$ days. All the others, save one which lived nearly 7 days, survived $8\frac{3}{4}$ to $9\frac{7}{8}$ days. The average is 10.7 days, but means less even than usual on account of the small number of animals.

In the course of the last five years, we have tested extracts obtained by different methods from the adrenal cortex with the view of determining whether they could substitute for the adrenals and thus prolong the life of adrenalectomised dogs and cats. During four or five years preceding this period we made numerous tests with cortical extracts upon adrenalectomised rabbits and guinea pigs. Various solvents were employed. Among the methods employed was precipitation by various salts and subsequent extraction of the precipitates, fractional precipitation, dialysis, etc. We did not obtain favorable results with the salt precipitates in the relatively small number of animals on which they were tried at that time. In view of the statement of Hartman and his associates (1928) that the life of adrenalectomised cats can be prolonged by subcutaneous administration of extracts obtained by precipitation with sodium chloride, we have made experiments to test this statement. Everything was done precisely in the way described by these observers, including control of the pH (in the case of the acetic acid extract electrometrically, and of the final product colorimetrically and electrometrically). The preparation employed by us was that designated by them as VIIIa. This represents the extract from which they obtained the best results. We shall refer to this extract as a salt precipitation extract. Fresh material was prepared twice a week. The dosage was the same as administered by them.

Table 4 contains results on 32 adrenalectomised cats treated subcu-ıneously with extracts of cortex of cattle prepared in this way. One of ıe cats (166-2) was in advanced pregnancy at the time of the first adrenal

TABLE 4

drenalectomised cats treated subcutaneously with extracts made by salt precipitation

RECORD NUMBER	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED		BEGAN TO REFUSE FOOD	ADRENAL WEIGHTS		PANCREAS CONGESTION	REMARKS
	First operation	Second operation					Left	Right		
	kgm.	*kgm.*	*days*	*days*	*hours*	*days*	*gram*	*gram*		
63–3	3.4	3.4	15	7	5	6			+	Small gastric ulcers
63–4	3.2	3.2	15	$7\frac{3}{4}$		6			+	
63–5	1.5	1.7	29	$8\frac{3}{4}$		$7\frac{1}{2}$			++	
63–6	3.2	3.2	15	$7\frac{2}{3}$		7			+	
63–7	4.1	4.0	15	$9\frac{1}{2}$		8			+++	
63–8	3.6	3.6	28	$17\frac{2}{3}$		$15\frac{1}{2}$		0.90	+++	Adrenals calcified
63–9	3.2	3.1	28	$8\frac{3}{4}$		$7\frac{1}{2}$			++	Ulcers (pylorus)
64–0	2.2	2.4	28	$6\frac{2}{3}$		6			+	
64–1	2.4	2.5	28	$23\frac{5}{6}$		20			+++	
64–2	2.5	2.8	28	$15\frac{1}{2}$		13			++	Ulcers (stomach)
64–8	2.9	2.9	42	8	$5\frac{1}{2}$	4	0.23	0.30		
64–9	3.9	3.6	42	$7\frac{2}{3}$		6	0.35	0.24	++	
65–0	3.7	3.8	42	$31\frac{1}{2}$		29	0.22	0.23	0	Old cat
65–3	3.0	3.3	44	$7\frac{5}{6}$		5	0.16	0.20	++	Ulcer (pylorus)
65–4	3.1	3.3	44	8	0	3	0.15		+++	
65–9	2.4	2.3	34	$8\frac{2}{3}$		$6\frac{1}{2}$	0.11	0.10	+++	Early pregnancy at first operation
66–0	2.8	2.7	34	$13\frac{1}{2}$		12	0.23	0.25	++	
66–1	3.1	2.9	34	$6\frac{1}{2}$		4	0.15	0.17	++	Ulcer (pylorus)
66–2	2.5	2.1	60	23	0	22	0.17	0.16	+++	Advanced pregnancy at first operation
67–0	2.9	2.8	15	$8\frac{1}{2}$		8	0.17	0.16	+++	
67–2	3.7	3.8	15	7	4	$5\frac{1}{2}$	0.24	0.22	+−	
67–3	3.3	2.9	15	$6\frac{1}{2}$		$5\frac{1}{2}$	0.16	0.16	++	Small erosion (stomach)
67–4	2.8	3.0	15	$13\frac{1}{2}$		12	0.12	0.12	+++	Ulcers (pylorus)
67–6	1.9	2.1	15	12	$1\frac{1}{2}$	11	0.10	0.12	++	
67–7	2.9	2.7	15	$11\frac{1}{2}$		8	0.23	0.27	+−	
67–8	2.9	3.0	15	$17\frac{1}{2}$		$15\frac{1}{2}$	0.12	0.17	++	
67–9	2.9	3.0	19	$4\frac{2}{3}$		4	0.26	0.33	+++	Small erosions (stomach)
68–0	3.5	3.5	19	7	$23\frac{1}{2}$	7	0.30	0.37	+++	
68–5	3.2	3.1	28	12	0	$11\frac{1}{2}$	0.14		++++	Ulcers (pylorus)
68–8	2.5	2.3	28	$6\frac{2}{3}$		$5\frac{1}{2}$	0.12	0.12	+	
68–9	2.3	2.1	28	$14\frac{1}{2}$		$13\frac{1}{2}$	0.16	0.14	++	Ulcer (pylorus)
69–0	2.9	2.7	28	$7\frac{1}{2}$		$6\frac{1}{2}$	0.15	0.16	+++	Ulcers (pylorus and duodenum)

All the cats were male except 164–0, 164–1, 164–2, 165–9 and 166–2.

In 163–8, 165–9 to 166–2 and 167–6 to 168–0 inclusive the left adrenal was removed first; in the others the right.

ɔeration and 18 days thereafter gave birth to 4 kittens. She lived 23 days ter removal of the second adrenal, the gland having been excised 42 days ter delivery. At autopsy the uterus was found incompletely involuted

and corpora lutei prominent. It is not known whether the pregnan y (and heat) exercised any effect upon the duration of survival, but le animal did not reach the maximum survival period seen in our cont ol cats (Rogoff and Stewart, 1929). We have considered it fair to place t is cat in table 4 in spite of the possible influence of the pregnancy so as o give the method being tested the benefit of any doubt.

Cat 165-9 was in early pregnancy at the time of the first operati 1. Three days after the operation she miscarried and gave birth to 5 embr s $2\frac{1}{2}$ to 3 cm. long. The second adrenal was removed 34 days after the fi st operation. The animal lived $8\frac{2}{3}$ days.

If the table is compared with our control tables for cats (Rogoff a d Stewart, 1929) it is seen that there is no essential difference. The ma i-mum survival period in table 4 is $31\frac{1}{2}$ days. This was in an obviously d cat. Although there may be some reason to suspect that very old c s

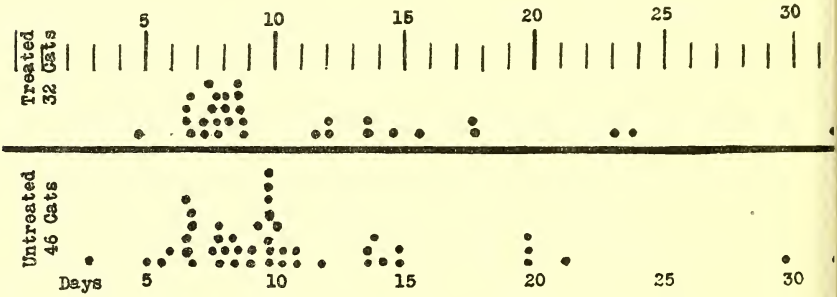

Fig. 1. The upper part of the chart represents the survival periods of our 32 c s (table 4) treated by subcutaneous administration of cortical extract prepared y precipitation with sodium chloride. The lower part of the chart shows the survi l periods of our 46 control, untreated cats.

and dogs bear loss of the adrenals better than younger animals this is ı t certain, and it would have been unjustifiable to exclude the animal fr ı the table on this ground.

The maximum survival period ($31\frac{1}{2}$ days) is exactly the same as in t control tables, from which two old castrated males were excluded. One f these lived slightly over 35 days, the other only $7\frac{1}{2}$ days. In table one cat reached $23\frac{5}{6}$ days. Among the controls one cat lived $29\frac{3}{4}$ da , and one-eighth of all the animals survived about 20 days or longer. table 4 three, i.e., one-tenth of the cats lived 20 days or longer. Amo the controls more than one-fifth lived 2 weeks or longer, and amo the treated cats less than one-sixth survived 2 weeks or longer. Betwe one-third and one-fourth of the treated cats lived 13 days or longer, a one-fourth of the controls did likewise. About three-eighths of the treat animals survived 9 days or longer, as compared with more than fo

sevenths of the controls. The average for the 32 treated cats was 11.1 days; for the 26 male controls 11 days; and for the 20 non-pregnant female controls $10\frac{3}{4}$ days. It is obvious that in every particular the results on the cats in table 4 treated with extracts by salt precipitation are the same as those in the untreated control series. The influence of the treatment was therefore completely negative.

The practical identity of the results in our treated cats and in our control series of untreated, adrenalectomised cats is shown very clearly in figure 1. The upper part of the graph is plotted from table 4 of this paper and the lower part from tables 1 and 2 of paper VIII. The numbers represent

TABLE 5

Adrenalectomised male dogs treated subcutaneously with extracts made by salt precipitation

RECORD NUMBER	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED		BEGAN TO REFUSE FOOD	ADRENAL WEIGHTS		ALIMENTARY CANAL		PANCREAS CONGESTION	REMARKS
	First operation	Second operation					Left	Right	Blood	Congestion		
	kgm.	*kgm.*	*days*	*days*	*hours*	*days*	*gm.*	*gm.*				
162–8	7.3	7.8	114	$10\frac{2}{3}$		8	0.47		++	++	+++	Ulcers (pylorus and duodenum)
164–6	8.2	9.0	67	$4\frac{1}{2}$		$4\frac{1}{2}$	0.62		+	++	++++	Ulcers (pylorus)
165–6	10.4	10.4	72	$4\frac{2}{3}$		3	0.66	0.70	+	++	+−	Ulcers (stomach)
166–5	9.2	9.4	60	$3\frac{2}{3}$		$3\frac{2}{3}$	0.53	0.54	+++	++	++	
166–7	8.6	8.7	34	$6\frac{2}{3}$		4	0.60	0.44	0	+	++++	Ulcer (duodenum)
166–8	11.3	10.6	34	11	$3\frac{1}{2}$	$9\frac{1}{2}$	0.55	0.49	+++	++++	++	Ulcers (stomach)
168–2	7.2	6.7	22	$8\frac{2}{3}$		7	0.41	0.43	0	+	+++	Erosions (pylorus)
168–3	11.4	11.2	22	$10\frac{3}{4}$		$9\frac{1}{2}$	0.65	0.61	0	0	+++	
169–1	9.8	10.1	21	12	4	9	0.79	0.78	++	++	++++	
169–2	8.7	8.7	21	$9\frac{2}{3}$		1	0.57	0.50	+	+	++	Ulcers (pylorus and duodenum)

In 165–6, 168–2 and 168–3 the left adrenal was removed first; in the others the right.

the days the animals lived after the second adrenalectomy. In both series the great bulk of the points lie between 5 and 10 days; a smaller number, between 10 and 15 days; one or two, less than 5 days; one or two, about 30 to 32 days. It must be remembered that the lower (control) series contains about 50 per cent more animals than the upper (treated) series.

The routine diet for cats (both control and treated) consisted of milk in the afternoon of the day of operation, the adrenalectomy being done in the forenoon. Milk was usually given also the day after the operation. Thereafter, fish or boiled meat on alternate days with raw meat. The boiled meat was in the form of a stew with potatoes, etc. Milk was given when the other food was refused.

Table 5 contains the results of subcutaneous injection, into dogs, f extract made by salt precipitation. None of the dogs lived as long as t maximum period of the controls. Four lived 10 to 12 days. Three liv less than 5 days. The average was about $8\frac{1}{4}$ days for the 10 anim comprised in the table. The result is therefore entirely negative.

In table 6 are comprised 12 dogs treated intravenously with the sar material. One of the dogs lived 25 days, 5 hours, and three lived about 2

TABLE 6

Adrenalectomised male dogs treated intravenously with extracts made by salt precipitation

RECORD NUMBER	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED		BEGAN TO REFUSE FOOD	ADRENAL WEIGHTS		ALIMENTARY CANAL		PANCREAS CONGESTION	REMARKS
	First operation	Second operation					Left	Right	Blood	Congestion		
	kgm.	*kgm.*	*days*	*days*	*hours*	*days*	*gm.*	*gm.*				
162–3	14.2	13.1	91	$13\frac{3}{4}$		$12\frac{1}{2}$	0.87		+	++	++++	Ulcers (pylorus)
162–4	6.0	6.8	81	8	$3\frac{1}{2}$	$7\frac{1}{2}$	0.42		+++	+++	+++	Wt. 6.5 kgm. n end
162–6	11.6	11.4	50	11	1	10			0	++	++++	
163–1	19.2	17.5	49	11	21	$9\frac{1}{2}$			+++	+++	++++	Large ulcers (d denum)
163–2	11.7	10.2	84	5	12	4		0.70	++	++	++	Large ulcers (sto ach and d denum). Wt. kgm. at end
164–5	10.5	9.5	40	$13\frac{2}{3}$		$13\frac{1}{2}$			++	++	++++	
165–8	13.3	12.6	41	25	5	$24\frac{1}{2}$	0.88	1.19	++	++	++	
166–6	11.3	11.4	34	$7\frac{2}{3}$		6	0.62	0.52	+++	+++	+++	Ulcers (pylorus)
166–9	9.6	8.9	34	$9\frac{2}{3}$		6	0.62	0.55			++++	Blood in stools. 8.5 kgm. at end
169–4	7.5	8.0	21	11	5	$9\frac{1}{2}$	0.43	0.45	+	++	++++	Stomach much co gested
169–7	11.3	11.2	20	14	1	$11\frac{1}{2}$	0.66		+	+	++++	Stomach blood cl and small erosio
169–8	8.0	9.2	20	11		10	0.72	0.77	+	+++	++++	Small ulcers (sto ach)

In 163–1, 163–2, 165–8 and 169–4 the left adrenal was removed first; in the others the right.

weeks. Of the 12 dogs in the table, eight, or two-thirds, survived 11 da or longer. The average for the 12 animals was 12 days. The results a decidedly inferior to those obtained with extracts prepared according our method and exhibited in table 1. But although the number of dogs too small to be quite satisfactory, we might interpret the results as slight positive. It does not seem unlikely that extracts which probably conta only a relatively small proportion of interrenalin should exhibit some pow of prolonging life when injected intravenously, even if the results subcutaneous injection were negative.

Accessories. It is self evident that great care must be taken in all work involving adrenalectomy to determine whether accessories are present, which can be seen and removed at operation or are only discovered at autopsy. In dogs, as we have already pointed out, accessories are quite rare but this is not so in cats. We have determined the frequency with which accessories were found in a large number of cats. In one group of 55 cats there were 5 in which an accessory was found and removed at the time of the operation. In another group of 66 cats there were two in which accessories were discovered and removed at operation. In the 121 cats there were seven with accessories, or about 6 per cent. This anatomical fact can be applied to test the thoroughness of the search for accessories in large series of cats. For instance, in a recent paper (Hartman, et al., 1928) describing experiments on a very large number of cats it is not stated that accessories were found in any of them although "in animals which survived a week or more careful post-mortem search was made for the presence of accessory cortical bodies." According to our statistics, it may be assumed that a certain number of the animals in this series possessed accessories. If this is so, the few cats which are stated to have survived beyond the longest survival period in our control, untreated cats, may have done so in virtue of the presence of accessories and not in virtue of the potency of the extracts with which they were treated.

Ulcers. We mentioned some years ago (Rogoff and Stewart, 1926) the frequency of gastric and duodenal ulcers in adrenalectomised dogs. In adrenalectomised cats ulcers are very common. In 46 control adrenalectomised cats there were ulcers in the stomach or duodenum or both in 14 animals. In 32 cats treated subcutaneously with extract prepared by salt precipitation there were 11 with ulcers. In 7 pregnant adrenalectomised cats there were 4 with ulcers. Altogether in 89 cats there were 29 with ulcers. That is, about one-third of the animals had ulcers. In a previous paper (Rogoff and Stewart, 1929) it was stated incidentally that ulcers in the stomach are more common in cats than in dogs after adrenalectomy. The proportion in dogs was given in an earlier paper (Rogoff and Stewart, 1926) as at least one-sixth of the cases. Larger experience and perhaps more careful search have shown that the proportion is higher than this in dogs and that there is much the same incidence in the two kinds of animals. For instance, in 118 adrenalectomised dogs, 48 had ulcers, about two-fifths of all the cases. The ulcers in the majority of cases extend through the mucosa. They are often quite large. Sometimes they are superficial, and these are usually small and multiple. The larger ulcers are also often multiple. Sometimes the large ulcers appear to have been formed by the confluence of smaller ones, giving the appearance of an irregular outline. The larger ulcers more frequently extend into or through the muscular coat, and not seldom only the peritoneum remains

unperforated. Occasionally they have perforated the peritoneum. In cats ulcers seldom extend as deeply into the muscular coat. In 32 unoperated cats, which were used for other experiments or died of causes other than loss of the adrenals, we found an ulcer in the stomach in only one animal. The great frequency of ulcers after adrenalectomy points to the same conclusion as the other pathological changes in the alimentary canal described by us in earlier papers, that the loss of the adrenals is apt to be associated with serious derangement in the gastro-intestinal tract.

SUMMARY

In paper V of this series proof was given that extracts of adrenal cortex from dogs (interrenalin) can prolong the period of survival of dogs after adrenalectomy beyond the maximum seen in control untreated animals. It was stated further that similar results were obtained in dogs by intravenous injection of cortical extracts from sheep's adrenals. The data on the influence of these extracts are given in the present paper. Subcutaneous administration of the extracts did not exert as favorable an influence as intravenous injection.

Extracts prepared from the adrenal cortex of cattle in the manner recently described by Hartman and his co-workers and administered subcutaneously, as in their experiments, were found to have no influence on the survival period of adrenalectomised cats, as compared with our untreated controls. These extracts, injected intravenously into dogs, were not nearly so effective as our own extracts. If they produced any definite effect at all, it was slight. Subcutaneous administration in dogs had no favorable influence upon the survival period.

Accessories were found in about 6 per cent of 121 cats operated on for removal of the adrenals.

Ulcers in the stomach or duodenum, of adrenalectomised dogs and cats, are very common. Of 118 adrenalectomised dogs, 48, or two-fifths of the cases, had one or more ulcers. Of 89 adrenalectomised cats, 29 had ulcers (about one-third of the cases). Of 32 normal cats, only one had an ulcer (in the stomach.)

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BLOOD PRESSURE FOLLOWING ADRENALECTOMY

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In the course of other investigations we have had an opportunity to make some studies on the blood pressure, in rabbits and dogs, following extirpation of both adrenal glands. Our experiments with rabbits have already been published elsewhere (Rogoff and Dominguez, 1924). Two dogs were used for comparing the normal blood pressure with that in rabbits. Later, these dogs were subjected to double adrenalectomy and blood pressure observations continued up till the death of the animal.

Previous reports on the blood pressure of adrenalectomized animals, made by a number of investigators, consist of observations made in acute experiments on anesthetized animals. Trendelenburg (1914) made some studies on cats, utilizing the method employed by Gärtner (compression of the leg and gradual release of pressure until the pallor of the pad begins to disappear) which permits blood pressure measurements to be made without the use of an anesthetic. He removed the adrenal glands in one- and two-stage operations and permitted the animals to recover from the immediate effects of the operation before making the blood pressure observations. The blood pressure, after double adrenalectomy, remained normal for twelve hours or longer, only sinking shortly before death. While the method employed by Trendelenburg permitted blood pressure measurements to be made in non-anesthetized cats, his animals did not survive long enough to have fully recovered from the effects of the operation for removal of the adrenals.

Anesthesia, operative procedures and other conditions capable of influencing the blood pressure render the above mentioned acute experiments of little value. Nevertheless, the better ones among them show that no significant alteration of the pressure occurs as the result of acute suppression of epinephrin secretion from the adrenal glands. This is to be expected in view of the well-established fact that epinephrin secretion from the adrenals is not indispensable for life and health (Stewart and Rogoff, 1917, 1919). It is sometimes argued that the extracapsular chromaffin tissue may suffice to furnish the necessary epinephrin in the absence of the adrenal secretion, but substantial evidence that this tissue secretes epinephrin is lacking. Its presence does not suffice to prevent death when most or all of the cortical adrenal tissue is removed, nor does it prevent the terminal fall of blood pressure in animals dying of adrenal insufficiency. The low blood pressure observed in Addison's disease, if primarily due to disturbed adrenal function, is more probably associated with deficiency of cortical function than with the epinephrin secretion.

Since acute suppression of adrenal function is apparently without effect upon the blood pressure, it is of interest to determine whether or not the

ressure is altered in adrenalectomized animals that have fully recovered rom the operation and are surviving a relatively long time. We found o significant change in the blood pressure, up to the time when the animal as obviously moribund, in 19 rabbits that were subjected to bilateral xtirpation of the adrenal glands (Rogoff and Dominguez, 1924). Of hese, 3 died in the first week (4, 6, 7 days), 8 in the second week (8, 8, 0, 10, 11, 11, 12, 12 days), 3 in the third week (15, 16, 16 days) and 2 in he fourth week (22, 26 days). One survived 89 days and two about 18 onths. During the course of the observations, one of the longest sur- ivors was mated and delivered a litter of 4 (dead) rabbits; at autopsy an ccessory cortical adrenal body was found in this animal.

Observations on dogs. The blood pressure was measured, in the dogs as the rabbits, by Van Leersum's (carotid loop) method. Details of the rocedure (on rabbits) are described elsewhere by one of us (Dominguez, 924). Briefly stated, the method consists of transposing a carotid artery nto a flap of skin so that it forms a loop on the neck of the animal. Meas- rements are made by adjusting a small cuff around the loop and inflating to compress the artery. The cuff is connected with a mercury ma- ometer, and the measurements can be made by auscultation or palpation f the artery beyond the cuff.

Both dogs were non-pregnant females. The adrenal glands were excised n two operations, an interval of about six weeks intervening between the moval of the right and left glands. Blood pressure measurements were corded before, during and following the removal of each adrenal. One nimal survived nearly twelve days, the other nearly thirty-seven days. he blood pressure observations were made daily, under relatively con- ant conditions and always by the same observer. In neither animal id the blood pressure fall below the levels observed before adrenalectomy, ntil the dog developed the usual symptoms that precede the moribund ate.

Condensed protocol. Dog, female; record number 120–5.

November 25, 1925, prepared left "carotid loop" (thyroid artery ligated). De- mber 22, 1925, to February 1, 1926, systolic blood pressure measurements made by lpation, thereafter systolic and diastolic measurements were auscultatory. April , skin of loop is inflamed (under surface); blood pressure measurements tem- rarily discontinued. June 15, weight 10.5 kgm.; right adrenal excised under ether esthesia (weight 0.680 gram); loop healing well. June 25, resumed blood pressure asurements. July 9 to 29, measurements discontinued (owing to illness of ob- rver). During this period the animal was in rut.

July 31, weight 11.1 kgm.; left adrenal excised under ether anesthesia (weight 50 gram). During the operation systolic pressure alone was measured (by palpa- on). 11:12 am., surgical anesthesia, blood pressure 152–140 mm. 11:14 a.m., 2–143 mm. 11:15 a.m., incision made; blood pressure 128–130 mm. 11:21 a.m., pulse ry feeble, rate 152; blood pressure about 80 mm. 11:23 a.m., adrenal removed; pulse eble. 11:25 a.m., wound closed; pulse rate 128. 4:30 p.m., systolic blood pressure

180–190 mm. (verified by a number of auscultatory observations); pulse rate 88. Post-operative recovery very good but animal is not yet in usual playful mood.

August 1, excellent condition. August 2, during observation she was annoyed by flies and became excited; pulse rate went up to 200 and the pressure rose from 135 to 160–170 mm. August 17, up to the present she has been in excellent condition. This morning she vomited a small amount of frothy fluid containing a live worm. At noon she ate a good meal. In the afternoon she vomited a small amount, otherwise appears to be in excellent condition. August 22, condition very good; played actively for a long while. August 31, condition unchanged; ate large portion of meat. September 1, seems less active; not as playful as heretofore and runs about less lively than usual. Not eager for food but ate her entire meal in the course of the afternoon. September 2, quiet, not playful; ate entire meal, little by little, during the day. September 3, condition about the same as yesterday; ate her meal of meat but did not care for the bones. September 4, seems apathetic; ate less than half of her meal of meat. In the afternoon she vomited some mucus and partly digested meat. September 5, 1:00 p.m., asthenic, body temperature subnormal; ate none of her meal; passed small amount of tarry feces. Systolic blood pressure 100–110 mm., diastolic below 40 mm. Hg. 5:00 p.m., passed some more tarry feces; is quite asthenic; pulse rate, 34. 8:45 p.m., condition poor; pulse rate, 34; vomited bile-stained mucus; died during the night.

September 6. Autopsy, 10:00 a.m. Subcutaneous fat in usual amount. Thymus large and fleshy. Thyroids normal in appearance, left gland larger than the right and parathyroid in left lobe reddish (the left thyroid artery was ligated when the loop was made). Heart and aorta normal. Carotid in loop normal. Striae in iliacs (normal). Lungs normal. Liver, spleen and kidneys moderately congested. Pancreas congested. Stomach contained bile and blood-stained fluid, mucosa hemorrhagic; small clots and four small erosions in pylorus. Duodenum, contents bile and blood-stained, mucosa moderately congested, small erosion near orifices of pancreatic ducts. Jejunum, contents same as duodenum, becoming more bloody lower down; mucosa moderately congested. Ileum, contents bloody, especially in the lower portion; mucosa congested in upper portion, quite hemorrhagic lower down. Large bowel contained some tarry fecal matter; mucosa and that of the rectum moderately congested. Uterus thick and boggy. Ovaries, small, containing yellowish bodies.

The long period of survival of this animal is explained by the fact that the second adrenal was excised toward the end of the period of rut. It has been shown (Rogoff and Stewart, 1927) that pregnancy and rut exert a protective influence in dogs deprived of both adrenal glands. The blood pressure observations, on this animal, are illustrated in figure 1. Measurements were not made during the operation for removal of the first adrenal because at that time the skin of the carotid loop was somewhat inflamed. During the second adrenalectomy the blood pressure fell considerably when the gland was being isolated for removal. Excessive anesthesia may have been partly responsible for this, but what is probably of greater importance, in explaining the fall of pressure, is that in this case the gland was in very intimate contact with the neighboring nerve structures. However, the animal made an excellent post-operative recovery and within a short time the blood pressure returned to normal.

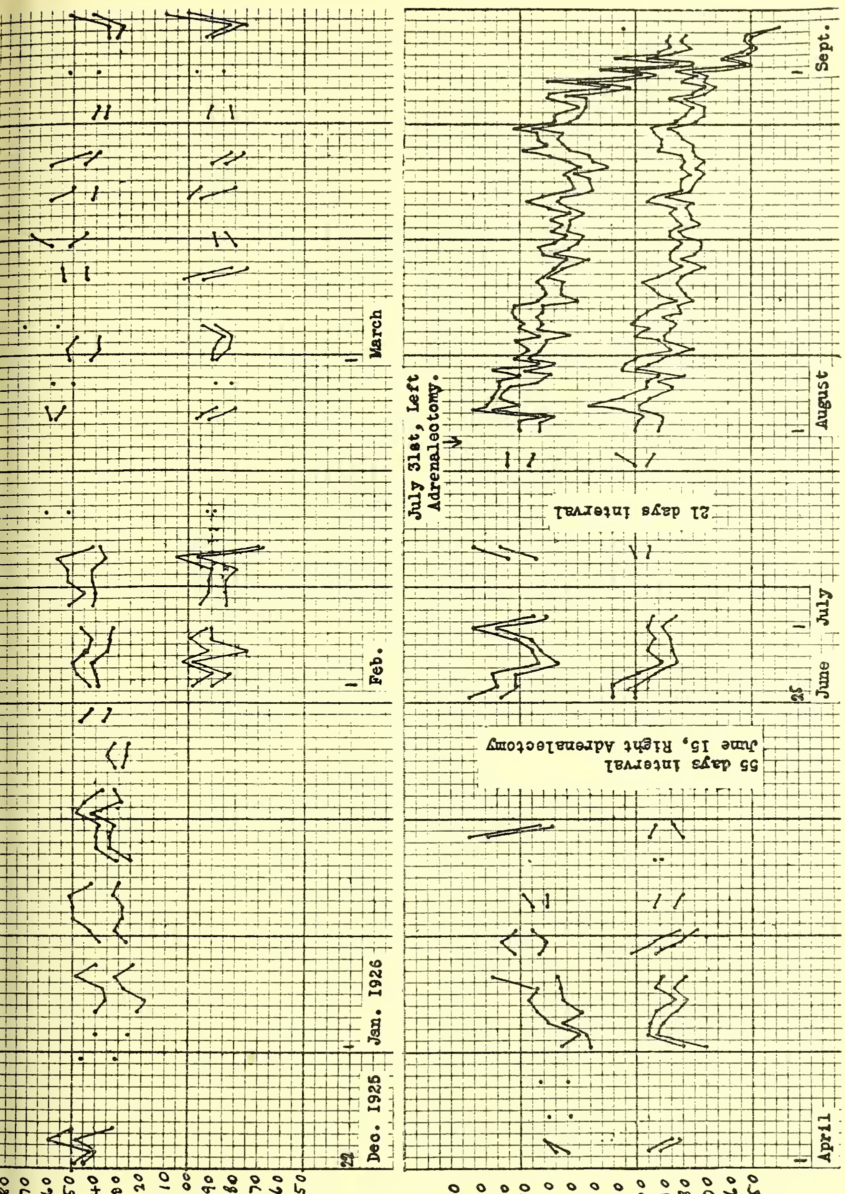
Dec. 1925
Jan. 1926
Feb.
March
April
June
July
August
Sept.
July 31st, Left Adrenalectomy.
21 days interval
55 days interval
June 15, Right Adrenalectomy
180
170
160
150
140
130
120
110
100
90
80
70
60
50

Fig. 1

Superficial inspection of the blood pressure curve in figure 1 may lead to the impression that following the removal of the second adrenal the pressure gradually declined. This, however, is mainly due to the fact that the first few observations after the operation were in the highest levels recorded. On closer inspection, it will be seen that the fluctuations in the balance of the curve were within the same range of pressures observed prior to the excision of the first adrenal, e.g., during January and the first part of April. The first significant fall of systolic blood pressure occurred about six days and of the diastolic pressure about five days preceding the death of the animal, i.e., about a month after extirpation of the second adrenal.

Condensed protocol. Dog, female; record number 120–6.

January 5, 1926, prepared left "carotid loop" (thyroid artery not ligated). May 11, blood pressure measurements, by auscultation and palpation, begun. June 12, between sixth and seventh observations she was disturbed by a fly; the systolic blood pressure rose from 140 to 150 mm., the diastolic from 96 to 105 mm. Hg. June 14, dog panting, room temperature 29.2°C. during the period of observation.

June 15, weight 8.6 kgm.; right adrenal excised under ether anesthesia (weight 0.720 gram). During the operation only systolic measurements were made. 2:17 p.m., incision made; successive blood pressure readings, 147, 148, 152, 154 and 156 mm. Hg. 2:21 p.m., 172 and 172 mm. Hg. 2:24 p.m., 182, 174, 175 and 175 mm. Hg. Adrenal removed. 2:29 to 2:36 p.m., 149, 153, 138, 152, 159, 148, 146, 144, 144, 156, 158, 154 and 156 mm. Hg. 2:36 to 2:40 p.m., 148, 144, 142, 142, 145, 140, and 140 mm. Hg. Wound closed. 143, 143, 145, 150 and 148 mm. Hg. 4:15 p.m., 174, 175, 174 and 174 mm. Hg.

June 16, pulse rate somewhat irregular during measurements. June 18 to July 29, blood pressure measurements temporarily discontinued to permit healing of slight ulceration of the skin on under surface of the loop. July 31, weight 8.3 kgm.; left adrenal excised under ether anesthesia (weight 0.770 gram). 10:37 a.m., surgical anesthesia; blood pressure readings 208, 200 and 195 mm. Hg. 10:42 a.m., incision made; 185, 182, and 188 mm. Hg. 10:43 a.m., 202, 198 and 198 mm. Hg. 10:46 a.m., 200 mm., adrenal massaged, 190 and 190 mm. Hg. 10:47 a.m., 188, 188 and 185 mm. Hg. 10:49 a.m., 168, 170 and 178 mm. Hg. 10:51 a.m., adrenal removed, 158, 156 and 150 mm. Hg. 10:52 a.m., 160 and 158 mm. Hg. 10:54 a.m., 160, 170 and 176 mm. Hg. 10:56 a.m., 158 mm., more ether administered, 164 mm. Hg, wound closed. 10:57 to 11:00 a.m., 150, 158 and 144 mm. Hg. 4:40 p.m., 162, 170 and 170 mm. Hg.

August 1, condition excellent. August 2, room warm, temperature 28.3°C. during blood pressure observations. August 6, condition, up to the present, very good; eating well and behaving normally. August 7, beginning asthenia and anorexia; blood pressure falling; she happened to be irritated by a fly and the blood pressure, which was already low, mounted from 84 to 98 mm. Hg (systolic) and from 60 to 68 mm. Hg. (diastolic). August 8, asthenic; total anorexia. August 9, has developed clonic twitching of the head. August 10, total anorexia; clonus of head less pronounced; asthenic. August 11, total anorexia and marked asthenia; vomited bile and mucus; clonus of head no longer present; died during the night.

August 12. Autopsy. Subcutaneous fat abundant. Thyroids normal. Thymus, small petechiae in lower portion. Lungs and heart normal. Kidneys normal in size; medulla congested; capsule strips with difficulty and tears renal substance; on section shows conspicuous yellowish straight tubules; glomeruli not visible. Uterus

rmal. Left ovary, one yellow body in upper pole; right ovary, a number of yellow dies. Stomach contains bile; mucosa congested. Entire small intestine contains ood and the mucosa is markedly congested, increasing from the lower portion of the odenum downward to the cecum. Cecum and appendix, mucosa intensely consted. Colon and rectum less congestion. Pancreas congested. Liver slightly ngested.

The blood pressure measurements, made on this dog, are recorded in ble 1, giving the minimum and maximum pressure in each set of observaons. As a routine, each set of observations, in both animals, consisted ten successive measurements, the average, in the table, representing e arithmetical mean of the ten measurements. No reading was disrded. During the operation for extirpation of the adrenal gland the acheal respiratory sounds in the anesthetised animal rendered auscultary observations impossible and measurements were made by palpation ly.

No significant alteration in the blood pressure, that might have been tributed entirely or chiefly to the loss of the adrenal, occurred during e operation for removal of the first or second gland. Just before excision the second adrenal the gland was purposely massaged, with the fingers, see if this would cause a rise in blood pressure but none was observed. ne pressure was somewhat high at the time, however, and it is probable at with a lower pressure an effect might have been obtained, since it is own that massage of the adrenals can liberate sufficient epinephrin to use a rise in blood pressure (Stewart, Rogoff and Gibson, 1916).

Following the removal of the second adrenal there was no change in the ood pressure during the period that the animal remained in good health, ., for about six days. It began to decline with the onset of anorexia and ntinued to fall as the terminal symptoms developed. The clonus of the ad that was present for about three days preceding death rendered the ood pressure measurements rather difficult and the diastolic (auscultary) observations somewhat unreliable or impossible. When the clonic itching was not present, it always recurred as soon as the carotid was cluded to make a measurement.

These observations indicate that in dogs, as in rabbits, extirpation of e adrenal glands does not result in any significant alteration of blood essure during the period of good health. This period, in dogs, may be week or longer in males, and in non-pregnant females that are not in t (Rogoff and Stewart, 1926), permitting observations to be made when e animal has completely recovered from the effects of anesthesia and om the surgical procedure. Decline of the blood pressure occurs as a rminal event, associated with other symptoms that have been shown to cur in dogs dying of adrenal insufficiency. It will not be profitable to eculate regarding the primary cause of this terminal fall of pressure,

TABLE 1

Blood pressure observations, dog, record number 120–6

DATE	TIME	BLOOD PRESSURE						PULSE RA[TE]
		Systolic			Diastolic			
		Maximum	Minimum	Average	Maximum	Minimum	Average	
		mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*per minu[te]*
May 11	10:00 a.m.	162	150	155.0	110	98	103.4	160
May 19	4:00 p.m.	162	152	156.9	112	93	103.5	128
May 20	5:30 p.m.	179	150	164.5	100	93	96.5	160
May 23	1:00 p.m.	152	138	142.5	98	88	92.1	144
May 26	4:30 p.m.	153	148	149.9	103	96	100.1	104
June 9	4:00 p.m.	145	135	139.2	105	92	97.3	144
June 10	5:00 p.m.	145	128	136.5	90	84	88.3	136
June 12	2:00 p.m.	150	139	143.9	105	95	97.2	144
June 14	4:20 p.m.	129	124	125.8	86	75	79.7	136
June 15	2:15 p.m.	Right adrenal extirpated						
	4:15 p.m.	175	174	174.2	125	123	124.0	124
June 16	10:00 a.m.	158	142	150.0	110	100	103.9	120
	5:40 p.m.	162	155	158.0	106	102	105.4	104
June 17	10:00 a.m.	156	152	154.5	111	110	110.1	88
	5:20 p.m.	147	140	142.8	105	98	101.0	86
June 18	10:40 a.m.	145	140	142.4	105	92	97.7	88
July 29	4:45 p.m.	146	144	144.9	112	100	103.3	152
July 30	5:36 p.m.	146	139	142.0	110	102	104.4	120
July 31	10:42 a.m.	Left adrenal extirpated						
	4:40 p.m.	170	162	167.3	130	130	130.0	112
August 1	2:20 p.m.	148	142	143.6	120	115	117.0	104
August 2	10:15 a.m.	162	158	160.8	135	120	124.1	108
	5:20 p.m.	150	142	145.0	100	92	96.2	88
August 3	9:55 a.m.	153	146	149.2	117	110	113.0	96
	5:30 p.m.	152	140	147.6	130	115	118.0	104
August 4	10:15 a.m.	140	120	131.1	110	90	100.8	128
	5:20 p.m.	148	140	142.9	110	94	102.7	112
August 5	10:15 a.m.	140	122	132.8	110	90	98.3	128
	5:15 p.m.	132	124	129.9	104	94	98.9	128
August 6	10:30 a.m.	121	102	112.6	87	76	82.9	128
	4:35 p.m.	110	95	101.8	82	78	80.6	152
August 7	10:40 a.m.	98	76	87.0	70	58	63.5	140
	4:10 p.m.	92	75	85.0	70	54	61.1	148
August 8	12:30 p.m.	100	92	95.2	68	60	63.5	120
August 9	4:35 p.m.	103	88	99.8				136
August 10	9:40 a.m.	89	70	79.2				136
	4:20 p.m.	90	78	84.1	53	50	51.0	128
August 11	10:00 a.m.	98	88	91.5				124
	3:50 p.m.	77	70	72.7				120

ut it may be assumed that suppressed epinephrin secretion has little or othing to do with it, and that it is associated with the phenomena that esult from loss of cortical adrenal function.

SUMMARY

Blood pressure measurements were made before, during and following drenalectomy, in dogs, by Van Leersum's (carotid loop) method which ermits observations to be made on non-anesthetized animals. Nearly ll the observations included systolic and diastolic measurements.

Observations, made daily, on one dog surviving double adrenalectomy bout twelve days and another about thirty-seven days, showed no sigificant change in blood pressure during the period of good health of the nimals. A decided and progressive fall of pressure occurred a few days efore death, with the onset of the terminal symptoms. This is in accord ith our earlier studies made on rabbits.

The dog that survived nearly thirty-seven days was operated for removal f the second adrenal toward the end of the period of rut.

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Vol. 90, No. 2, October, 1929

ROGOFF, J. M. and G. N. STEWART (CLEVELAND). **Functions of the Adrenal Glands.**

It has been well established that the function of the epinephrin secretion from the adrenals is not indispensable. The important function of the glands consists of the elaboration and probably secretion of a hormone by the cortex. To distinguish this hormone from adrenalin and to indicate its origin in the interrenal tissue, we have employed the name, "Interrenalin." Marked prolongation of life has been observed by us in adrenalectomised animals when extracts of adrenal cortex were administered. Beneficial influence has been obtained, in a number of cases of Addison's disease, by administration of these cortical extracts.

Reprinted from THE AMERICAN JOURNAL OF PHYSIOLOGY
Vol. 93, No. 2, June, 1930

Adrenalectomy and muscle fatigue. H. C. STEVENS and J. M. ROGOFF.

Experiments were made upon decerebrate, adrenalectomised cats, in which the fatigue curve of the gastrocnemius muscle was studied. Curves obtained during the period of survival in good health, following double adrenalectomy, did not differ materially from curves obtained with animals that were not subjected to adrenalectomy. The behavior of cats during and following decerebration, the blood pressure and respiratory response, the specific reactions of the gastrocnemius muscle as evidenced by the fatigue curve, the component phases of the twitch myogram and the chronaxie of the tibial nerve and the muscle in the adrenalectomised cats were compared with similar observations, made under the same conditions, in control non-adrenalectomised animals.

Reprinted from The American Journal of Physiology
Vol. 97, No. 3, June, 1931

On the nervous mechanism of epinephrin secretion. J. M. Rogoff, P. Wasserman and R. Hoecker.

The relatively constant rate of epinephrin liberation from the adrenal glands, under ordinary experimental conditions, was found to be an average of about 0.00025 mgm. per kgm. of body weight per minute, in a large number of cats and dogs, when determined by measuring the epinephrin concentration in adrenal vein blood collected at a known rate of flow. This spontaneous (normal) liberation of epinephrin has been shown to be governed by a nervous mechanism situated in the upper dorsal region of the spinal cord. Hemisection of the cord in this region results in suppression of epinephrin discharge from the ipsilateral adrenal and transection affects both glands (Stewart and Rogoff, 1917, 1920).

When the functional integrity of the brain is interfered with, beyond the effects of ordinary anesthesia, (e.g., by compression, mechanical destruction or by anemia caused by ligation of the arteries to the brain) the rate of epinephrin secretion from the adrenals is within, but usually in the upper limit of, the normal range. This could be explained by the possibility of removal of an inhibitory influence of a mechanism in the brain, permitting the spinal cord mechanism to exercise its maximum influence. Experimental evidence has been obtained to support this view.

If the rate of epinephrin liberation is determined, in an animal under ordinary anesthesia and again after interference with the brain, the initial rate is usually found to be somewhere near the above mentioned average, while the rate in the second instance is at or near the maximum of the "normal" range (Rogoff, 1924). Additional experiments have been performed. In these the animals (cats) were decerebrated at various levels. It was found that transection of the anterior part of the cerebrum does not materially alter the rate of epinephrin secretion, but decerebration in the region bounded by the superior colliculus and the optic chiasm results in the same degree of increase in the rate of epinephrin liberation from the adrenals as was observed in the other experiments already mentioned. If decerebration is followed by shock, the epinephrin output may fall to a level considerably lower than the initial rate.

These experiments may have a bearing upon the observation that, in certain experiments, strychnine may cause *a preliminary fall* followed by the usual large increase in the rate of epinephrin secretion (Stewart and Rogoff, 1919). It may also be suggested that if two opposing mechanisms are concerned in the liberation of epinephrin from the adrenals, this may explain the great difficulty in demonstrating reflex changes in the epinephrin secretion. This, and other, possibilities are reserved for further study.

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3692

Pressor Effect of Guanidine Salts on the Non-anesthetized Rabbit.

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A possible relationship between guanidine intoxication and essential hypertension has been suggested.[1] It was of interest to study the question in non-anesthetized animals. This report contains the results of daily examinations for blood pressure and pulse rate in 12 experiments performed on 5 rabbits provided with a good carotid loop (Van Leersum). The salts used (Eastman Kodak Co.) were methylguanidine nitrate (9 experiments), methylguanidine sulphate (2 experiments), and guanidine nitrate (1 experiment). All the rabbits received 0.1 gm. per kilo of body weight but in different concentrations, 1:10 and 1:20 in distilled water, and 1:20, 1:30 and 1:50 in salt solution (4 and 8 per 1000). The total volume of fluid injected was from 3.7 to 8.2 cc. of distilled water and from 6 to 18 cc. of saline. The weights of the rabbits varied from 2.945 to 4.125 kg. After several weeks of observation, the animals received one of the guanidine salts, injected slowly intravenously (marginal vein of the ear). Blood pressure, pulse rate and general reactions were carefully observed immediately after the injection and daily thereafter.

The behavior of blood pressure and pulse rate, in one of the animals, is shown in Fig. 1. Blood pressure is expressed in mm. Hg. (scale to the left); pulse rate in beats per minute (scale to the right). The space between two vertical lines corresponds to one half day. Blood pressure values are the mean of 10 consecutive readings. After the first injection, A, the blood pressure fell and the pulse rate increased, in marked contrast to their behavior before the injection. This condition lasted 18 days after the injection. At

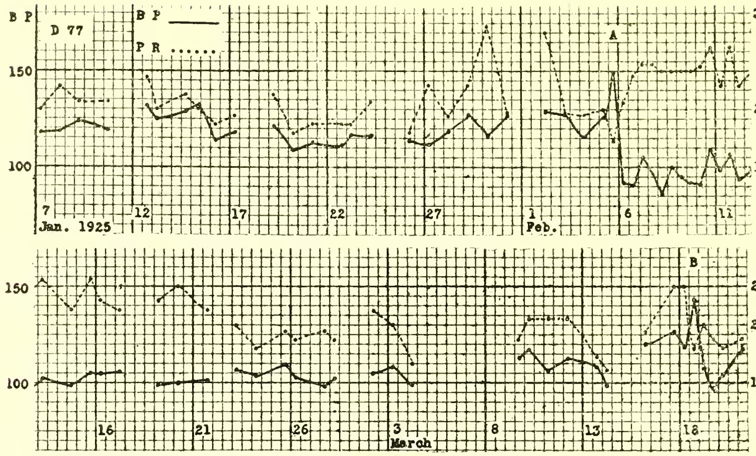

Figure 1.

B, another injection of same amount of same salt (8.4 cc. of 1:20 in 4:1000 saline) had a similar effect immediately following the injection, but practically no after effect. The graph after this point is similar to the portion before the injections, and has been omitted. The graph under B is a good example of most of the experiments. In a few instances the dip of the pulse rate is more pronounced, reaching 128 per minute, in a few others the peak of the blood pressure is higher.

In one animal the effect after injection was more pronounced than shown in A. The blood pressure oscillated about 80, the pulse rate about 300. The animal died on the fifth day. At autopsy a marked dilatation of the heart was found, hydrothorax (bilateral) and ascites. There is little doubt that methylguanidine produced in this rabbit a severe injury of the myocardium.

The observations may be summarized thus:

1. In no instance did blood pressure reach 170 mm. Hg., *i. e.*, within normal limits.[2, 3] The highest reading was 165 mm. The highest averages were: 150-159 mm. Hg, 2 instances; 140-149, 7; 130-139, 0; 120-129, 3. The last three are negative as far as blood pressure is concerned. Two of these negative results (in the same animal) show that with this dose the pressor effect of methylguanidine may be absent. The other occurred with guanidine nitrate. De Waele and Bulcke have already said that: "Chez le lapin, même à hautes doses, la guanidine reste sans effet sur la pression sanguine et sur le vague."[4]

2. The pulse was slowed in all cases even when the blood pressure was not greatly altered. Slowing of the pulse rate is, in these experiments, more conspicuous than in Alles's report.[5] My results with

methylguanidine salts are comparable to his, and, in a few instances more pronounced than the effect he obtained with ethylguanidine. The difference may be accounted for by the urethane anesthesia and the smaller doses of methylguanidine used by him. Practically all workers agree that this circulatory effect of guanidine salts is independent of the vagus.[1, 4, 5]

3. The time relation of blood pressure and pulse rate effects cannot be made as precise with the method I have used as with a continuously recording device. I find that the highest blood pressure and the lowest pulse rate coincided in 8 out of 12 experiments and that in the remaining 4 experiments the pulse rate effect preceded the rise in blood pressure. The slowest pulse rate was recorded as early as 5 minutes and as late as 49 minutes after the injection. The rise in blood pressure was recorded as early as 8 minutes and as late as 2 hours and 10 minutes after the injection. The blood pressure curves had a single peak in 7 experiments, and a plateau in 5; this plateau was observed to extend for from 35 to 272 minutes. The pulse rate curve had an inverted peak in 8 experiments; and a low flat part in 4, the latter extending in an interval of time varying from 26 to 95 minutes. The blood pressure rise lasted more than the slowing of the pulse in 5 instances, about the same in 4, and less in 3.

4. The other signs of intoxication occurred as follows: salivation (11 instances out of 12 experiments), jerks of the extensors of neck (8 instances), diarrhea—soft feces (7), clonic spasms of legs (6), dyspnea, occasionally violent (4), tremor of lips (3). Of these signs, salivation was first to appear and diarrhea the last to disappear.

5. In a subsequent experiment, part of the graph being reproduced, the injection was repeated when the blood pressure rise of the first injection was beginning to subside. Immediately after the second injection the animal became very weak, the blood pressure rose again (maximum 165 mm. Hg.) without any slowing of the pulse rate, and the respiratory movements became very frequent. Half an hour later the blood pressure was 132 mm. Hg., pulse rate 284 per minute, and dyspnea violent. Eighty minutes after the second injection the animal went abruptly into collapse. The pulse was imperceptible, respiratory movements progressively weaker. The animal died 5 minutes later.

6. The circulatory effect of the methylguanidine salts used is inconspicuous when compared with the general picture of the intoxication.

7. One animal died with all the signs of circulatory embarrass-

ment due to myocardial weakness, as shown at the autopsy by dilatation of the heart, hydrothorax and ascites.

8. A toxic theory of essential hypertension would be more plausible if a substance were found which causes, in normal animals, persistent elevation of blood pressure above normal limits, without obvious signs of general intoxication.

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[1] Major, R. H., and Stephenson, W., *Bull. Johns Hopkins Hospital*, 1924, xxxv, 186, 140.

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[3] Dominguez, R., *J. Exp. Med.*, 1927, xlvi, 443.

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THE SYSTOLIC BLOOD PRESSURE OF THE NORMAL RABBIT MEASURED BY A SLIGHTLY MODIFIED VAN LEERSUM METHOD.

FINAL REPORT.

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In this report it is intended to complete and extend my previous publication (1) on the systolic blood pressure of the rabbit. An account of the method used, together with the technique both of the loop operation and of the measurements, is to be found in the first report and will not be given here.

The number of loops made has been 186, the first one on April 4, 1923, the last one on July 30, 1926. Good loops, that is, even, soft and of convenient length, were obtained in 97 (52.2 per cent), of which 4 were lost before any measurement was made (trauma, pneumonia) and 3 are omitted now because the measurements were started in the course of an experiment. The majority of failures is due to necrosis or infection of the loop. From March 31 to April 16, 1925, 23 loops were made, of which only 3 were satisfactory (13 per cent), 18 being spoiled by necrosis or infection (78.3 per cent). On the other hand, from September 25 to September 30 of the same year, 22 loops were made, of which 16 were good (72.7 per cent), only 5 being ruined by infection or necrosis (22.8 per cent). On July 29 and 30, 1926, 7 loops were made, all good. These figures show the extremes of failure and success which have been met in this work. The success of the other series falls between 50 and 60 per cent, so that the poor result with the loops made in April, 1925, seems to have been due to some extraneous factor not determined. The other causes of failure are general, such as pneumonia, pleurisy, otitis media, etc., and are not peculiar to this plastic operation. There is still another complication which may

TABLE I.

No. of rabbit	Sex	Observation period	No. of days on which blood pressure was taken	Mean of blood pressure 1st day	Maximum blood pressure whole period	Minimum blood pressure whole period	Pulse rate 1st day	Fastest pulse rate whole period	Slowest pulse rate whole period	Body weight		Remarks
										Initial	Final	
		1923		*mm. Hg*	*mm. Hg*	*mm. Hg*				*kg.*	*kg.*	
37-2	F	May 15–Sept. 12	92	126	165	102		272	172	2.110		Weight Apr. 4. Snuffles
39-6	F	May 15–June 1	11	125	136	90						Snuffles. Ear canker
39-7	F	May 16–June 5	15	130	170	125						Snuffles
42-0	F	July 25–Oct. 17	48	118	147	90	228	264	176		2.275	Albino. Litter of 10 Aug. 1
42-1	M	Aug. 3–Sept. 10	31	103.6	132	72	204	224	120	2.675		Weight June 5
46-1	M	Aug. 4–Oct. 17	40	104.1	130		208	256	160	2.390	2.600	Constriction. Snuffles
46-9	M	Oct. 16–Dec. 3	35	136.6	157	105	232	264	160	2.350	2.390	White Angora
		1923–24										
47-0	M	Aug. 4–Jan. 16	103	102.4	149	90	194	248	128	2.375	2.805	
		1923										
47-3	M	Aug. 18–Aug. 20	2	129.5	143	119	204	264	200	1.702		Weight July 24. Trauma
47-4	M	Oct.–Dec. 1	37	105.9	138	82	248	264	112	1.895	1.905	
47-7	M	Oct. 18–Nov. 26	31	101	133		162	236	136	2.120		Weight Oct. 27. Constriction. Abscess at angle of left lower jaw
48-2	M	Oct. 9–Dec. 1	44	111.9	129	75	242	264	156		2.060	
		1923–24										
48-3	M	Oct. 8–Oct. 13	266	109.5	144	77	222	280	112	2.290	3.145	Sciatic injury, Oct. 18, 1923
		1923										
48-4	M	Aug. 21–Oct. 12	16	114.1	127		240	300	208			Resistance. Abscess (flank). Constriction

48-5	M	Oct. 18–Dec. 1	35	139.5	156	92	253	296	160		2.120	Sciatic injury
48-7	M	Aug. 15–Dec. 1	58	128.1	172	103	296	300+	192	2.285	2.610	Albino. Mediastinal abscess during experiment
48-8	F	Aug. 14–Sept. 8	23	110	143	94	156	240	148		2.585	Abscesses in abdominal wall
		1923–24										
48-9	M	Aug. 14–Oct. 13	301	134.5	201	116	248	300+	144	2.890	3.015	
		1924										
D 1	M	Jan. 7–Feb. 26	44	99.2	135		200	220	136	2.310	2.855	Constriction
D 3	F	Jan. 7–Feb. 11	31	126.8	140	95	200	280	132	2.290	2.000	Purulent pleurisy
D 6	F	Jan. 7–Feb. 26	45	128.1	140		176	232	152	2.065	2.495	Constriction, marked
D 8	F	Jan. 7–Mar. 27	39	144.8	160	103	192	300+	176	2.795	2.610	
D 10	F	Jan. 7–Feb. 26	44	140.4	150		264	280	188	2.465	2.705	Constriction
D 12	F	Jan. 7–Feb. 26	44	129.3	132		224	224	152	1.970	2.435	Albino
D 13	M	Feb. 1–Mar. 27	46	138.7	157	95	192	240	136	2.345	2.275	
D 16	F	Apr. 15–Apr. 29	10	136	159	124	240	256	216	2.275	2.305	
D 19	M	Apr. 15–Apr. 29	10	143.6	162	125	232	256	184	2.060	2.050	
D 21	F	Apr. 15–Apr. 29	9	154.6	162	135	216	216	176	2.175	2.200	
D 22	F	Apr. 15–June 17	19	126.7	168	111	192	232	176	2.280	2.480	
D 23	F	Apr. 15–May 15	12	115.3	133		184	216	144	2.080	2.150	Constriction
D 24	M	May 1–May 15	10	121	129		208	232	168	1.845	1.915	Constriction
D 25	F	May 20–July 17	28	84.6	110	72	216	248	160	1.840	2.085	Resistance
D 26	F	May 1–May 28	21	128.2	148		200	220	168	1.975	2.225	Constriction
D 27	F	Apr. 22–May 20	20	122.9	135		216	240	184	1.815	2.000	Constriction
D 28	F	June 13–July 11	23	109.9	131	91	168	224	144	2.055	2.310	
D 31	F	Apr. 22–May 20	18	108	119		176	224	160	1.920	1.975	Constriction
D 33	M	Apr. 23–Apr. 28	5	109.6	120	87	200	224	168	1.885	1.865	Trauma
D 34	F	May 30–June 17	16	125.6	133	97	184	200	136	2.370	2.635	
D 41	M	June 12–July 10	23	137.1	145	96	192	232	164	2.325	2.290	
D 42	M	June 12–July 10	22	108	120	90	224	240	192	1.820	1.965	
D 45	M	June 5–July 11	29	107	122	89	208	240	152	2.125	2.060	

TABLE I—*Continued.*

No. of rabbit	Sex	Observation period	No. of days on which blood pressure was taken	Mean of blood pressure 1st day	Maximum blood pressure whole period	Minimum blood pressure whole period	Pulse rate 1st day	Fastest pulse rate whole period	Slowest pulse rate whole period	Body weight Initial	Body weight Final	Remarks
		1924		*mm. Hg*	*mm. Hg*	*mm. Hg*				*kg.*	*kg.*	
D 46	M	June 12–July 11	23	135.1	149	105	184	232	160	2.184	2.115	
D 49	F	June 30–July 19	16	116.7	140	97	184	232	168	2.140	2.365	
D 53	F	June 30–July 19	17	95.6	116	89	208	252	176	1.970	2.050	
D 55	F	June 30–July 19	17	123.1	155	107	216	248	168	2.425	2.215	Litter of 3 July 6
D 56	M	June 30–Aug. 22	43	101.7	132	84	208	248	160	1.955	2.165	Ear canker
D 59	F	June 30–July 19	17	115.8	145	98	196	232	176	1.910	1.965	
D 61	M	Aug. 11–Sept. 30	15	95.8	131	90	224	256	208	2.095	1.705	Sciatic injury
D 62	F	July 8–Sept. 10	28	129.6	154	102	216	256	168	2.415	2.315	Litter of 8 July 16
D 65	M	Aug. 12–Sept. 10	25	138.2	170	112	192	240	168	2.150	2.325	
D 66	M	Aug. 11–Oct. 27	61	100	125	70	208	280	144	1.945	2.460	Large abscess in thigh
D 67		Aug. 1–Sept. 10	25	97.6	129	92	248	264	176	2.420	2.635	
		1924–25										
D 69	F	Sept. 25–Feb. 10	76	145	174	126	224	248	160	2.825	3.460	
D 77		Nov. 10–Feb. 5	40	120.4	140	98	200	288	152	2.815	3.580	
D 78	M	Nov. 10–Feb. 5	39	114.5	156	91	264	280	168	3.115	3.730	Resistance. Constriction
D 80	M	Nov. 10–Mar. 24	69	144.4	168		216	240	152	2.965	3.040	Otitis media. Constriction
D 82	F	Nov. 10–Feb. 10	43	110	135	90	224	248	168	2.325	3.355	
		1924										
D 83	F	Nov. 10–Nov. 29	6	113.1	130	110	248	256	144	2.725	2.875	Chewed loop

		1924–25										
D 84	F	Nov. 24–Feb. 10	35	121.1	152	106	248	296	216	2.780	3.320	
D 85	M	Nov. 24–Feb. 10	33	103.7	141	99	200	248	144	2.820	3.350	
		1925										
D 87	M	Jan. 8–Mar. 24	59	110.8	130	82	264	264	148		3.625	Weight Oct. 24, 1924, 2.270 kg.
		1924										
D 88		Nov. 24–Nov. 26	3	109.6	115	94	200	200	176	2.480		Death from diarrhea
		1925										
D 89		Jan. 8–Mar. 26	60	118	142	97	224	248	160		3.195	Weight 2.300 kg. Nov. 22, 1924
		1924–25										
D 91	F	Nov. 24–Feb. 14	38	128.5	142		216	224	168	2.220	2.945	Constriction
		1924										
D 92	F	Nov. 24–Nov. 25	2	121.1	125	102	288	300	288	2.320		Accidental death
		1924–25										
D 94	F	Nov. 24–Feb. 10	34	126.3	167	112	232	288	200	2.310	2.460	
D 96	M	Nov. 24–Mar. 24	62	114.5	152		240	272	184	2.150	2.880	Constriction
		1925										
D 97	F	Apr. 15	1	133.5	140	130	248			3.480		
D 98	F	Apr. 15	1	131.1	138	124	272			2.550		
D 116	F	Sept. 24–Oct. 30	17	124.4	133	107	224	256	216		3.010	Constriction
D 118	F	Sept. 24–Oct. 30	16	123.9	138	95	232	256	208		2.330	
D 119	F	Sept. 24–Oct. 30	16	100.6	122	92	264	288	224		2.670	
D 123	F	Oct. 19	1	84.3	90	80	240			2.250		Weight Sept. 25, 1925. Trauma
D 125	M	Oct. 19–Oct. 30	10	101.7	119	89	232	240	216		2.320	Constriction (late appearance)
D 127	M	Oct. 19–Oct. 30	10	122.9	134	85	232	248	200		2.160	
D 131	M	Oct. 19–Oct. 30	10	97.4	112	72	232	240	184		2.080	
D 134	M	Oct. 20	1	71.3	75	69	208			1.720		Trauma. Weight Sept. 29, 1925
D 135	F	Oct. 19–Oct. 30	10	73.4	89	68	184	232	184		2.590	
D 136	M	Oct. 19–Oct. 30	10	82	105	80	176	208	176		2.510	
		1926										
D 137	F	June 10–June 22	9	114.2	129	102	192	208	176		2.920	

TABLE I—*Concluded.*

No. of rabbit	Sex	Observation period	No. of days on which blood pressure was taken	Mean of blood pressure 1st day	Maximum blood pressure whole period	Minimum blood pressure whole period	Pulse rate 1st day	Fastest pulse rate whole period	Slowest pulse rate whole period	Body weight		Remarks
										Initial	Final	
		1925–26		*mm. Hg*	*mm. Hg*	*mm. Hg*				*kg.*	*kg.*	
D 138	F	Oct. 19–Apr. 22	103	91.9	112	77	216	280	192	2.475	2.650	Constriction
D 139	F	*1926* June 10–June 22	9	102.2	129	98	216	272	200		3.140	
D 140	M	*1925–26* Oct. 19–June 22	111	101.7	132	74	200	264	184	2.065	3.410	
D 141	M	Oct. 19–Apr. 22	92	91	126	79	208	280	184	1.885	3.060	Sciatic injury, Nov. 21, 1925
D 142	M	*1926* June 10–June 23	10	132.9	136	105	224	272	224		2.980	
D 143	F	*1925–26* Oct. 19–June 22	107	106.3	138	85	240	288	216	2.310	3.470	
D 144	F	*1926* Aug. 24–Oct. 4	10	100.7	115	90		240	208		2.565	
D 145	M	Aug. 24	1	90.4	92	89						Trauma
D 146	M	Aug. 24–Oct. 4	10	91.9	103	84	208	224	184		2.365	Accidental death
D 148	F	Aug. 24–Oct. 4	10	77.4	98	70	208	240	200		2.835	

appear when the animal kicks within the controlling box, and to which young animals, very quick in their actions and with soft bones, are specially susceptible. This is referred to below as "spinal trauma" and in Table I as "trauma." In some instances, under the same circumstances, instead of a complete paraplegia, there appears a partial

TABLE II.

Blood pressure	No. animals	Per cent
mm. Hg		
150–	1	1.1
140–149	6	6.7
130–139	13	14.4
120–129	20	22.2
110–119	18	20.0
100–109	16	17.8
90–99	10	11.1
80–89	3	3.3
70–79	3	3.3
	90	99.9

TABLE III.

Pulse rate per min.	No. animals	Per cent
280–299	2	2.3
260–279	5	5.9
240–259	13	15.3
220–239	17	20.0
200–219	29	34.1
180–199	13	15.3
160–179	5	5.9
140–159	1	1.2
	85	100.0

paralysis of one or both of the hind legs, without disturbance of the sphincters, and the animal recovers completely. This is referred to as "nerve injury." This complication is caused by the use of a controlling box and is not inherent in the loop method. One complication peculiar to the method is that some animals chew the loop. Five

did this: four (D 65, D 80, D 83 and D 96) during an interval in which no measurements were taken; and one (D 142) during a period of daily readings (March, 1927). The last one has not done it again so far, and three of the other four did not persist, so that in these animals the loop healed again and remained in good condition. But D 83 kept on chewing it until it became so scarred and so hard, that no more measurements could be taken. Eventually the animal died of hemorrhage. The reason for this is not clear, because repeated examination shows that there is no anesthesia or analgesia in the loop. Finally, one animal (D 25), on being lifted from the cage, caught its claw under the loop, kicked and tore it. This last accident (1 out of 97 good loops) and the "spinal trauma" are avoidable. The latter occurs in general on the first measurement and only in young animals, so it can be avoided by using full grown animals. This is preferable to packing the animal so tightly in the box that it cannot move. These are the only complications I have seen, which can be directly or indirectly attributed to the loop method. I have never seen thrombosis of the carotid or inflammation of the loop in animals under observation, which Van Leersum (2) reported having seen in one animal, or in fact any ill effects on the general condition of the rabbit. I have measured the blood pressure for as long as 15 months, with almost daily readings (1), and I am still measuring one rabbit 18 months after the first reading.

The data to be discussed now have been arranged in tabular form. They represent routine observations only, and not the results of any experimental condition imposed upon the animal, excepting of course the actual procedure of measuring the blood pressure and also a period of inanition of a few days to which several animals were subjected early in the work. Only one animal (No. 48-9) showed any effects of inanition, and the variations observed were not the extremes of the total variation recorded (1). On the other hand several of the animals developed diseases of various kinds, or were found to be pregnant or were the victims of some accident. These appear in Table I under "Remarks" and will be discussed later on. The blood pressure of a total of 90 rabbits (including the first 63 reported before) on the 1st day of observation is seen in Table II. The figures represent the arithmetic mean of the first 10 consecutive readings. The difference

between this table and the first one published (1) is partly due to better statistical treatment (a more careful distribution of the means). Three rabbits appear between 70 and 80 mm. Hg and none beyond 150 mm. The table, as it stands now, shows that on the 1st day of the examination, 60 per cent of the rabbits had a systolic blood pressure between 100 and 129 mm. Hg, and 85 per cent between 90 and 139 mm. Hg. The table, small as it is, compares in a general way with similar compilations made on men and women on the 1st day of examination. Concerning the published data on the blood pressure of the rabbit (direct measurement) it will be enough to quote the following: Volkmann (3) gives 90 and 108 mm. Hg. Meyer (4), after compiling the literature up to 1881, 51 experiments, comes to the following conclusion: "Dannach kann man als Grenzwerthe des mittleren Blutdrucks normalen Kaninchen ca. 70 und 140 mm. Hg annehmen." A remarkable conclusion, I think. Tigerstedt (5) says: "Beim Hunde schätzt man den Blutdruck auf 130–180 mm. Hg, beim Kaninchen auf 80–120."

The pulse rate of the rabbits reported in the present paper, counted on the loop itself at the end of each set of blood pressure readings, is shown in Table III. The pulse rate of the 1st day only has been given. By counting every other beat in a quarter of a minute, and multiplying by 8, it was possible to obtain the fast rates recorded. The error in these figures is at most ±8, small for the purpose. It appears that the pulse rate of 84.7 per cent of these rabbits ranged, during the first measurement, between 180 and 259 beats per minute. Tigerstedt (5) quotes the figures of Colin (1888), 120–150 per minute, and Ellinger (1894), 120–160 per minute, as the pulse rate of the rabbit. I have had no access to the original works quoted by Tigerstedt, so I do not know under what conditions Colin and Ellinger counted the pulse rate. But since it is much simpler to count the pulse than to measure the blood pressure, and since it can be done with very little disturbance to the animal, the pulse rate becomes an invaluable index of the presence of psychic or other factors which may have at the same time some effect on the blood pressure.

It is important to note that neither the mean of blood pressure nor the pulse rate of the *first* observation is in general the highest recorded during the whole period. Thus, for instance, only five

showed on the 1st day as fast a pulse rate as the fastest during the entire interval of observation of the respective animals (D 12, D 21, D 87, D 88, 48-7), and of these only one was near the maximum of the whole series (No. 48-7, 296 per minute), discarding of course those animals in which only one observation was made. With regard to the blood pressure, the number of animals in which the highest single reading of the corresponding period of observation was at most 10 mm. Hg higher than the mean of the 1st day, was sixteen, and of these eight were examined during less than 1 week. The remaining eight are D 10, 12, 21, 24, 34, 41, 116, 142. The distribution of the

TABLE IV.

Blood pressure	No. animals	Per cent
mm. Hg		
200–	1	1.2
170–179	4	4.9
160–169	7	8.5
150–159	10	12.2
140–149	15	18.3
130–139	22	26.8
120–129	12	14.6
110–119	7	8.5
100–109	2	2.5
90–99	0	
80–89	2	2.5
	82	100.0

maximum blood pressure by animals is seen in Table IV. By maximum is meant the highest blood pressure recorded *at least once* during the whole period of observation of the corresponding animal. Eight animals have been omitted because they were examined only during 1, 2 or 3 days. The table shows that on prolonging the observation period, 71.9 per cent reached 130 mm. Hg, 45.1 per cent reached 140, and 26.8 per cent reached 150, at least once, whereas on the first examination only 22.2 per cent were at or above 130 mm. Hg (see Table II).

I shall consider in more detail those which reached or passed 160 mm. Hg. D 8, D 19, D 21, D 65 and D 80 had only isolated high

figures, so that in their protocols there is no *mean* of 10 readings at or above 160. Nos. 37-2, 48-7 and D 94 had only one *mean*, and Nos. 39-7 and D 22 two *means* above 160 mm. Hg. D 69 showed *means* between 160 and 173, 11 times out of 76 (14 per cent), *scattered* throughout the period of observation. There remains one rabbit, No. 48-9, whose graph was reproduced in the first report (1). The number of *means* at or above 160 is 92, out of 516 (18 per cent), but what makes this observation noteworthy is the fact that, excepting scattered high means in October and December, 1923, practically

TABLE V.

Blood pressure	No. daily averages		Per cent	
	No. 48-3	No. 48-9	No. 48-3	No. 48-9
mm. Hg				
190–199		1		0.3
180–189		3		1.0
170–179		15		5.0
160–169		33		11.0
150–159		76		25.2
140–149		84		27.9
130–139	6	57	2.2	18.9
120–129	12	31	4.5	10.3
110–119	34	1	12.7	0.3
100–109	117		43.8	
90–99	79		29.6	
80–89	19		7.1	
Total.........	267	301		

all the readings made in September and the first third of October, 1924, were at or above 160 mm. Hg.

What is the behavior of the pulse rate? In general, when the blood pressure rises in a normal rabbit, the pulse rate increases, but the converse is not true: an increase in pulse rate is not necessarily acompanied by a rise in blood pressure. If the animal resists, whether the blood pressure rises or not, the pulse rate increases. In Table I the exceptional animals from the point of view of resistance are referred to by the word "resistance" under "Remarks." Under conditions of "excitement" there may be no effect on pulse rate or

blood pressure, or a marked increase in both or only in the pulse rate. Excitement is an unsatisfactory word to use, because it involves so much which is subjective. Without attempting to define it, I shall give a list of the observations included under this heading: tickling the animal's nose or closing its nostrils for a few seconds during a set of measurements; after a few days of inanition putting a piece of carrot under its nose, while measurements are being taken; placing adult male rabbits, after several months confinement, with females, both in heat and not in heat, and taking the blood pressure and pulse rate before and after copulation. Under these conditions "excitement" is expected to be produced, and the animals show, indeed, signs which can be interpreted as such. Yet the results on blood pressure or pulse rate are not consistent or uniform in all the rabbits examined. A few examples may be seen in the first paper (1). The meaning of "an increase in pulse rate" will be understood from an examination of the figures of the two animals which exhibited the highest blood pressure, D 69 and No. 48-9.

TABLE VI.

Pulse rate	Daily averages		Per cent	
	No. 48-3	No. 48-9	No. 48-3	No. 48-9
280–299		2		0.7
260–279	1	12	0.3	4.0
240–259		59	0.0	19.7
220–239	8	77	3.0	25.8
200–219	25	94	9.5	31.4
180–199	42	41	16.0	13.7
160–179	110	12	41.7	4.0
140–159	57	2	21.6	0.7
120–139	20		7.6	
100–119	1		0.3	
Total.........	264	299		

D 69, as stated above, showed 11 means between 160.2 and 173 mm. Hg (with a mean of the means of 165) and the pulse rate of the corresponding days was between 208 and 248 per minute (mean 229). No. 48-9 showed 29 consecutive means between 155 and 193 mm.

Hg, with a mean of the means of 174, and the pulse rate of the corresponding days varied between 212 and 272 (mean 245).

The totals of the data from Nos. 48-9 and 48-3 are seen in Tables V and VI. Their graphs may be seen in the first paper (1). They represent the limiting cases found in this investigation. A few animals had a lower blood pressure than No. 48-3, but they were examined for a much shorter time, or their pulse rate was higher, or their figures were more scattered in distribution, or their behavior was not quite so good. Again, no other animal had a higher blood pressure than No. 48-9. So that these observations help in determining what might be called the boundary conditions of experimental hypertension in rabbits.

It is not practicable to present all the data in detail. If ever a compilation on the "normal" rabbit were attempted (and such a compilation is an urgent need of laboratory workers),[1] then this material would find its proper place there. It is only necessary here to show the salient facts observed on which a criterion for a pathological rise in blood pressure in the rabbit may rest. It must be borne in mind, that by "normal blood pressure of the rabbit" is meant the systolic blood pressure of a healthy looking rabbit *under the conditions of the measurement.* The practice of taking the blood pressure of the rabbit a few days before the experiment, *as control,* has less value than is generally conceded. 1 week, or 1 month of observation, is not sufficient to characterize completely the blood pressure curve of a rabbit, and does not guarantee that the pressure will behave in the same way in a subsequent interval of time. Finally, the purpose of the investigation must be considered. For acute experiments, there is little need of a carotid loop. On the other hand, a method like this is indicated for the study of prolonged alterations in blood pressure, which may simulate either the clinical picture of hypertension or Addison's syndrome. With these reservations in mind, a criterion could be formulated as follows:

(*a*) Under the conditions of the measurement, a rabbit may be said to have a pathologically high blood pressure, if the blood pressure

[1] The recent work of Brown, Pearce and Van Allen is an excellent beginning in this direction (11).

oscillates *above 180 mm. Hg* and does not fall below that figure during a length of time dependent on the nature of the experiment.

(*b*) Under the same conditions, the blood pressure may be considered pathologically high if it oscillates *about 170 mm. Hg* with a concomitant pulse rate *below 200 beats per minute*.

The introduction of the pulse rate in (*b*) allows the avoidance of consideration of resistance, "excitement," and the like, and makes unnecessary the discarding of any figure in the course of the investigation. It seems superfluous to add that these criteria are provisional. Strictly speaking they rest on observations made on 90 "normal" animals (over 30,000 blood pressure readings, about 2,900 pulse rate counts), but they are reinforced by the subsequent experience with these animals under several pathological conditions. Since these observations, moreover, spread over 4 years, and since the animals were obtained from different dealers in different years, it is expected that future experience with the method here adopted will not differ grossly from that described in the present report. The observations of Van Leersum himself (1911, 1912) on twelve rabbits, agree very well with mine.

An Important Source of Error of the Method.—The following phenomenon has been observed in several rabbits.

D 138, Feb. 4, 1926, 3.09 p.m.* 100–101–79–82–84 = 93–98–98–100 = 103–97–100–99 = 102–101–103–103 = 103–92–92 (pulse rate 232).

D 80, Jan. 21, 1925, 10.55 a.m.* 152–102–112–108–112–118–126 = 141–132–132–136 = 146–143–143–145 = 145–143–142 = 141–142 (pulse rate 224).

Jan. 30, 1925, 11.36 a.m.* 144–140–140–138 = 139–139–140–105 = 102–108–116–120 = 130–131–130–135 = 130–132–128–132 (pulse rate 224).

* Cuff adjusted to loop.

This abrupt fall in blood pressure occurs at any time in a given rabbit. If observed once in an animal it may be observed many times. No sign of a more general character accompanies this phenomenon: no alteration in pulse rate, no change in behavior, no respiratory disturbance, no pupillary effect. It may probably be explained by a local constriction of the carotid under the influence of

the pressure applied on the cuff. The fact that it does not appear in all animals is not an objection to this explanation, because the carotid is subjected to this stimulus intermittently for a short time (3 to 5 minutes), and it is not to be expected that all carotids will react in the same way to a given stimulus. It is clear that the change must be local and not the expression of an actual fall of arterial pressure, which would be accompanied by a change of pulse rate. In Table I it appears under "Remarks" as "constriction." In some animals it is very slight, for instance:

D 78, Nov. 17, 1924, 3.18 p.m.* 138–132–133–132 = 132–130–96 = 129–128–127–127.

In others it is frequent and becomes disturbing, as in the following example:

D 6, Jan. 26, 1924, 10.27 a.m.* 115–110–104–89–88 = 112–113–115 = 114–112– (pulse rate 184).
114–112–113–97 = 106–112–106–108 = 112–116 (pulse rate 176).

*Cuff adjusted to loop.

I have discarded no animal on this account, but the "minimum blood pressure" has no meaning under these conditions, and has been omitted from the table. It is enough to recognize this phenomenon to avoid the error produced by it.

Changes in the Carotid within the Loop.—The majority of the carotids enclosed in a loop showed after the death of the animal a marked transverse striation of the intima. The striations are due to a slight thickening of the intima. Under the microscope the lesion is not well defined: a slight sclerosis of the subintimal tissues and here and there a moderate vacuolization of the muscular coat. This striation has been observed in the remaining parts of several carotids, a long time after the loop had been severed on account of necrosis; that is, in arteries to which *no cuff had ever been applied.* It has been found absent in arteries in which measurements had been taken for a considerable time. In a few animals which died immediately after the operation (possibly ether death), the examination of the carotid revealed sharp transverse lines of intimal tearing, very probably produced by stretching the artery during dissection. Before this injury was recognized and avoided, it was not uncommon to see, before the final suture of the loop, small extravasations of blood on the muscular layer of the carotid, which gave to the artery a slightly beaded appear-

ance, the more noticeable since the artery at the end of the operation contracts down through exposure. With more care in the manipulation of the artery this lesion may be reduced in extent, and possibly eliminated altogether. The fact is, the rabbit's carotid is a very thin and delicate object and must be treated accordingly. I think that the lesion observed in a good number of the carotids is due to the cicatrization of these intimal operative injuries.

Shapiro and Seecof (7), reviewing some of the methods of blood pressure measurement in the rabbit, say that Van Leersum's would be an excellent one "provided the artery could be thus isolated without altering the compressibility of its walls through inflammatory reactions. This objection seems to render the method both impractical and prohibitive." The authors fail to realize that the method is not sensitive enough to detect small changes in the thickness of the artery. The cuff effect in this method consists of several parts, the cuff proper (a piece of cotton fabric and of rubber tubing), the skin with and without hair, subcutaneous tissue and the vessel wall. Of these, the first three become softer and more supple in time, which, theoretically at least, would lower the value of the total effect, whereas the thickening of the intima is not progressive as far as I have been able to ascertain. Under these conditions, fluctuations of 40 mm. Hg *in one animal* several days, or several weeks apart, or occasionally in 1 day, cannot be due to variation in compressibility of the arterial wall. Finally, in experiments on arteriosclerosis (to be reported soon) where *calcification* was found in the media of the carotid within the loop, as well as in other large vessels, and in the aorta, no constant elevation of the blood pressure was observed.

MacWilliam in his review (8) on the blood pressure of man says: "It may be taken as established that high blood pressure readings, when carefully taken, represent approximately correct measurements of the actual intra-arterial pressures as a rule. It is only in a small minority of abnormal cases of thickened arteries with excessive tonic contraction, etc., that serious discrepancy may occur, sclerotic conditions without muscular contraction having no important influence. Digital compression for 3 or 4 minutes or massage of the artery are useful in removing abnormal resistance and have the advantage of not causing congestion of the limb which may arise from repeated compressions by the armlet." The lesion in the carotid of my rabbits is of the type considered by MacWilliam as having no important influence, and the method, as actually used, amounts to a digital massage of the artery, so that, if this massage were as efficacious as MacWilliam believes and such "excessive tonic contraction" were ever present in the rabbit, the method would automatically eliminate this source of error.

Blood Pressure in Pathological Conditions.—After what has been said before it will be easy to see the difficulty of attaching importance

to small variations in blood pressure, within the range of figures between 80 and 170 mm. Hg. Their importance increases as the pressure approaches these limiting values. The diseases that have occurred in animals under observation (not experimented upon, excepting the blood pressure readings) are, in order of decreasing frequency: coryza, ear canker, pneumonia (with or without pleurisy), purulent pleurisy, subcutaneous abscesses, meningitis (drooping and rotation of head). Diseases found at autopsy, not diagnosed during life: coccidial cysts in abdomen, otitis media (one or both sides, without meningeal involvement), scarred kidneys, arteriosclerosis of aorta (slight), pulmonary abscesses (discrete), pulmonary mycosis, mediastinal abscess. Accidental injuries: "spinal trauma," "sciatic injury," total infarction of kidney (ligature of renal vein during adrenalectomy).

Of all these conditions only two deserve special mention: "Spinal trauma" and meningitis. During the terminal coma of the latter the blood pressure is low, 70–80 mm. Hg. Occasionally, however, the pulse is slow and irregular, and the actual blood pressure readings are not reliable. Concerning "trauma," the observation is as follows: the animal is within the box, quiet; blood pressure is taken as usual. Suddenly the animal moves within. The motion may not appear to be of excessive violence. The blood pressure rises abruptly (140–150 mm. Hg) and the pulse is very slow and strong. Soon afterwards (1 minute or so) the pressure gradually falls and the pulse rate increases. Greater details cannot be given because the occurrence fortunately is rare and takes place within a very short time.

In the other conditions the blood pressure findings are discordant. In one rabbit suffering from a large unnoticed abscess of the thigh, the blood pressure was low (the graph has been reproduced in another paper (9)). During the course of pleuropneumonias the blood pressure may lie between 80 and 90 (see graph for No. 47–0 (10)), or between 90 and 110 mm. Hg. In No. 48-7 the mediastinal abscess, a large mass twice as large as the heart, situated on the right side, anteriorly, and adherent to the pericardium, showed a certain effect on the blood pressure, the more interesting since it was completely misunderstood during life. The observation is so unusual, that an abstract of the protocol is presented.

No. 48-7, albino, male rabbit. July 31, 1923, carotid loop is made, weight 2.440 kilos. Partial necrosis of distal portion of loop. Healed completely.

Aug. 15, first blood pressure readings (see Table I). Aug. 18– Sept. 8, blood pressure between 120 and 129 mm. Hg (74 per cent of daily means). Oct. 6 and Dec. 2, 1923, blood pressure between 130 and 149 (72.5 per cent of daily means.)

Dec. 3, lead carbonate smeared in carrot, fed by hand, about 25 mg. daily. Dec. 8, weight 2.550 kilos; Dec. 23, best weight, 2.675 kilos. Dec. 24, daily dose of lead carbonate increased to 78 mg.

Jan. 4, 1924, diminished appetite; Jan. 12, weight 2.480 kilos; Jan. 17, weight 2.190 kilos. Jan. 20, little appetite. Jan. 26, lowering of blood pressure; blood pressure from Dec. 3, 1923, to Jan. 24, 1924, between 120 and 139 (78.4 per cent of daily means). Feb. 6, lowest weight, 2.120 kilos.

The loss in weight and the lowering of blood pressure (see below) were thought to be due to lead carbonate, and the latter was interrupted until Feb. 18, 1924. From Feb. 18 to May 31, lead was given daily in increasing dose, from 40 up to 300 mg. Feb. 16, weight 2.260 kilos. Weight increases slowly in spite of increasing dose of lead.

Feb. 20, symptoms while drinking water, as if water had entered the trachea. Abundant râles. Bronchial (or tracheal) moisture persisted until Mar. 19. On Mar. 1, it was thought that the animal had a foreign body in the trachea. Apr. 11, weight 2.480 kilos; May 26, weight 2.285 kilos. June 2, animal looks sick. Blood pressure from Jan. 26 to May 31, 1924, between 100 and 119 mm. Hg (87 per cent of daily means). Concerning the pulse rate the only thing at all remarkable is the fast rate in the second half of May, between 240 and 280 per minute (mean 258). Blood pressure on June 2, between 74 and 78 mm. Hg, pulse rate 208. June 3, lies on side, does not move. June 4, dead.

The beginning of this disease may have been concomitant with the first fall in weight in the latter part of Dec., 1923, and the formation of a mass in the mediastinum (probably from lymph nodes) may account for the spell on Feb. 20 and the slow recovery from it. The increase in weight in spite of increasing dose of lead carbonate militates strongly against lead poisoning. Lead given in this fashion to a group of animals (of which No. 48-7 is one) has shown no deleterious action (experiments to be reported later). The animal was in excellent physical condition up to June 2, 1924. It had been mated repeatedly during the whole observation, the last copulation having occurred on May 20.

Carotid Loop in Dogs.—As a further check on the method, loops were made in two dogs. Here it was possible to use the stethoscope and find auscultatory criteria for systolic and diastolic pressure similar to those used in man. The daily means of one of the dogs ranged from 123 to 165 mm. Hg, 77.7 per cent lying between 130 and 149 (85 days), for the systolic pressure, and from 69.1 to 103 mm. Hg, 87 per cent between 80 and 99, for the diastolic. The pulse rate oscil-

lated between about 60 and 184 per minute, 87 per cent of the counts being between 80 and 139. The other dog was examined during a much shorter time: systolic between 125.8 and 164.5 mm. Hg (10 daily means), diastolic 80–103, pulse rate 104–160. Both these dogs were subjected later on to double adrenalectomy. A complete report will appear shortly in collaboration with Dr. J. M. Rogoff.

SUMMARY.

The blood pressure and pulse rate of 90 normal rabbits have been studied for various periods of time, from 1 day (accidental death interrupting the observation) to 15 months. The main data are presented in a table containing the blood pressure and pulse rate on the 1st day of observation, the maximum and minimum of both during the entire period of observation of each animal, together with the sex and weight of the animal. Separate tables are given showing the distribution of blood pressure, pulse rate and the "maximum" blood pressure by animals. Detailed data on two animals observed for the longest time are given in tabular form. The anatomical changes that occur in some carotids enclosed in a loop are described and discussed. Considerations on "excitement" and pathological conditions which arise spontaneously in rabbits are given. A criterion for a pathologically high blood pressure in rabbits is proposed.

The blood pressure of the normal rabbit ranges between 70 and 170 mm. Hg. The pulse rate, taken simultaneously with the blood pressure, fluctuates between 112 and 300 per minute.

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EXPERIMENTAL ATHEROSCLEROSIS AND BLOOD PRESSURE IN THE RABBIT.

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The relation between atherosclerosis and blood pressure has engaged the attention of several workers following the statement of Fahr (1) and Van Leersum (2) that rabbits fed on abnormal diet (egg, liver) develop a high blood pressure. The published material that I have examined is disconcerting, due not so much to the variety of methods used to determine the blood pressure, as to the lack of sufficient information concerning the "normal" fluctuations of blood pressure, that is, the fluctuations observed in normal animals with the particular method used. Van Leersum, who claimed to have found a marked elevation of blood pressure under the influence of a liver diet, obtained no lesion whatever in the circulatory apparatus of his rabbits. Again most workers seem to be under the impression that the blood pressure is a constant, that is, that if the blood pressure oscillates in a certain region for a few weeks before the experiment, then any increase above this region that may occur afterwards during any experimental condition is necessarily due to the experimental condition, although these values may be well within values recorded from other normal animals. I have had considerable experience with Van Leersum's method (3, 4) and have given curves which sufficiently illustrate the fallacy of that assumption. For instance, in the graph of Rabbit 48-3 (3) it may be seen that the blood pressure oscillated around 100 mm. Hg for fully 10 months (from November, 1923, to September, 1924) and then rose in September and October, reaching 140 (average) on September 27. If an experiment had been started in the last week of August and this rise had been observed, the observation would have been supported by a good control period, but an inaccurate conclusion could have been drawn. Another type of curve is shown here, and

still others may be found in former papers (3–5). To avoid repetition, by "blood pressure" and "normal range" is understood the systolic blood pressure of the rabbit as obtained from a carotid loop (Van Leersum's method) and the range of blood pressure in normal rabbits as determined previously by the writer.

The experimental atherosclerosis of the rabbit has in itself considerable intrinsic interest, so it seemed worth while to repeat the experiment. The literature on cholesterol feeding experiments is voluminous. For an introduction to the subject the reader is referred to the references given here, particularly Schönheimer. In the present work egg yolk was chosen since natural emulsions have been found best suited for the purpose. The rabbits (five in number) were given their usual food (hay, oats, greens) throughout the experiment; the yolk of one or two eggs was mixed with powdered unleavened bread and dried at 37°C., the whole appearing finally as yellowish crisp masses. The animals ate it readily in the beginning, but after some time they seemed to tire of it, so the yolk was diluted with plain water and the stomach tube used. The blood pressure was taken daily in the manner explained elsewhere (3). These five animals were chosen at random. One had been measured as a routine for several months, others for less time. Two of the five received lead carbonate by mouth in additon to the egg yolk. It seems justifiable to report the two cases where lead was also given, D 1, D 10, for the following reasons:

1. The association of lead poisoning and high blood pressure in man has always been considered in clinical medicine.

2. Lead carbonate in the form given to these animals and lead acetate given by stomach tube, in my experience, do not produce high blood pressure in the rabbit (unpublished data).

3. Recent work done in this country on the general subject of lead poisoning throws doubt on the efficiency of absorption of lead by the gastrointestinal canal (summarized in Reference 6).

4. There is no essential fact in the behavior or in the autopsy of these two animals that could be attributed with certainty to lead.

The curves were plotted at the completion of the experiment. The organs of the animals were carefully examined after death. Microscopic examination, however, was not systematically done. A brief analysis of Van Leersum's report (2) will be found at the end of the

paper, followed by a note on the results obtained by other methods of measuring the blood pressure.

The essential data on these five animals will be given in the form of condensed protocols.

D 1.—Male, brown, Belgian rabbit. Nov. 30, 1923, carotid loop is made; weight 2.310 kilos. Jan. 7, 1924, blood pressure measurements started; weight 2.625 kilos.

Feb. 26, feeding experiment began; one egg yolk mixed with powdered "Matzos" and 30 mg. of lead carbonate smeared in carrot and fed by hand, daily; weight 2.855 kilos. Mar. 13, daily dose of lead carbonate increased to 60 mg. Mar. 29, daily dose of lead carbonate increased to 80 mg. Apr. 6, best weight 3.360 kilos.

Apr. 14, conjugate motions of eyes and head, toward the right, with drooping of right ear. Lead and egg feeding are interrupted. Apr. 16, same condition; weight 2.660 kilos. Apr. 21, right ear is full of a foul smelling creamy pus; weight 2.410 kilos. Apr. 30, eyes were found to possess well developed corneal arcs. May 11, weight 2.395 kilos. May 17, death in coma.

Total number of yolks consumed, 40. Total amount of lead carbonate given, 2.690 gm.

Blood Pressure.—Highest pressure recorded was 135 mm. Hg on February 7. This animal was one of those which exhibit a phenomenon described in the preceding paper (4) and ascribed to a local constriction of the carotid under the stimulation of the external pressure applied on the cuff. It is well illustrated by the following examples.

Feb. 6, 1924, 10.21 a.m.* 121–71–101–112 = 115–126–125–128 = 125–125–120–123 = 128–124–122–120 = 121–122–124–127 (pulse rate 192).

Mar. 4, 1924, 10.09 a.m.* 103–88–68–0 (15 seconds)–99–92–93–93 = 96–95–93–95 = 76–69–70–89 = 95–93–94–91 = 92–91–97–95 = 95–95–96–93 (pulse rate 168).

* Cuff adjusted to loop.

This phenomenon renders the tabulation or plotting of the figures almost impossible. It appeared throughout the course of the experiment, but not every day. It was absent in the last part of the experiment, when the intracranial complication of the otitis media became evident. The blood pressure during this latter period was, in general, low, oscillating between 70 and 90. The pulse rate varied between

136 (February 23 and April 23) and 220 (January 17) per minute. During the terminal coma the pulse became very irregular, a few beats passing through at 90 mm. Hg.

Autopsy.—Heart: base of large mitral cusp infiltrated with fatty substances. Aorta: large patch of infiltration at opening of arch branches, extending for a short distance into the common root of carotids and into left subclavian. In ascending arch there are a few minute nodular elevations. Nothing in thoracic aorta. In abdominal aorta, several small nodules and two streaks, one at root of celiac trunk, another at level of renal arteries. These two streaks are perpendicular to the axis of the aorta. Remainder of aorta and iliacs, normal. Pulmonary artery shows several elongated patches of moderate size, along posterior wall, parallel to the axis. Left adrenal, 950 mg.; right 860 mg. (weighed on March 26, 1927, in formol in the interval, see discussion). Corneal arcs, bilateral, well formed. Brain: white, thick, purulent exudate at level of tentorium cerebelli, both sides of midline. Right middle ear is filled with a creamy pus.

D 10.—Female, brown rabbit. Dec. 6, 1923, left carotid loop is made; weight 2.280 kilos. Jan. 7, 1924, blood pressure readings started; weight 2.465 kilos.

Feb. 26, feeding experiment began; one egg yolk mixed with powdered "Matzos" and 30 mg. lead carbonate smeared in carrot and fed by hand, daily; weight 2.705 kilos. Mar. 7 and 12, best weight 2.840 kilos. Mar. 13, daily dose of lead carbonate increased to 60 mg. Mar. 26, animal looks sick; weak; egg and lead are withheld. Mar. 27, weight 2.555 kilos. Apr. 16, egg given again; animal has recovered its former appearance and behavior, but not its weight; weight 2.515 kilos. Apr. 26, weight 2.700 kilos.

May 1, egg given through stomach tube; lead carbonate given again, 50 mg. daily. May 2, no corneal arc in either eye. May 15–17, lead carbonate suspended in egg yolk emulsion, stomach tube. May 16, weight 2.350 kilos. May 18, dead.

Total number of egg yolks consumed, 55. Total amount of lead carbonate given, 1.780 gm.

Blood Pressure.—Highest figures recorded before experiment: 146 (January 7, 1924), 150 (January 23), 149 (February 25) with averages for day, 140.4, 140.3, 144.8 respectively. Highest figures recorded during experiment: 152 (March 5), 150 (March 6), 149 (March 11), 148 (March 12), 150 (March 15) with averages for day, 139.6 (30 readings), 143.2, 145.9, 140.6, 143.0 respectively. From March 26 to April 10, the blood pressure reached the lowest level observed during

the whole experiment, as low as 87 mm. Hg. This is the same period in which the animal appeared sick, concomitantly with loss in weight, loss in appetite, and, as it will be seen afterwards, increase in the pulse rate. I have no explanation for this. I have seen nothing like it in my experiments with egg yolk alone, or in the other animal which received lead together with the egg, or in several animals which have had lead carbonate or lead acetate alone (unpublished data). The pulse rate during this period oscillated between 216 and 288 per minute. The contrast in the behavior of pulse rate and blood pressure in the three periods, before March 26, from March 26 to April 10, and after April 10, is best seen in tabular form, where I have taken figures corresponding to the two extremes and mean of pulse rate for the respective periods.

Date	Pulse rate per min.	Blood pressure
1924		*mm. Hg*
Jan. 7	264*	138–146
Feb. 5	264	119–130
Feb. 9	272	118–124
Feb. 25	280	139–149
Jan. 9	232	116–138
Jan. 22	240	110–130
Mar. 17	232	130–135
Feb. 11	188	101–116
Mar. 26	216	87–92
Mar. 31	264	98–107
Apr. 5	280	97–101
Apr. 7	288	93–103
Apr. 16	192	102–110
May 1	144	111–126
May 14	240	122–129

* These fast rates are counted by groups of two $\frac{\downarrow\ \downarrow}{1}\ \frac{\downarrow\ \downarrow}{2}\ \frac{\downarrow\ \downarrow}{3}$.

With increasing rates the pulse becomes very rhythmic and this process of computation is accordingly easier.

Autopsy.—Aorta is mottled throughout with yellow spots and streaks, slightly elevated, parallel to the axis of the vessel; somewhat more abundant in arch and thoracic portions. For a distance of 1.5

cm. above the opening of the celiac trunk these small infiltrated areas become confluent. Infiltration from this point downward is less and less marked. Pulmonary artery shows a large, irregular, slightly raised patch, at bifurcation, extending both ways for a short distance. In left kidney there are a few yellow streaks in outer zone of pyramid. Eyes show no visible corneal arcs. Adrenals are large, right weighs 440 mg. (weighed on March 26, 1927, almost 3 years in formaldehyde solution; left adrenal has been split open and a piece of central portion cut off for microscopic examination; there was no obvious difference in size at the time of the autopsy).

D 6.—Female, brown rabbit. Dec. 4, 1923, carotid loop is made; weight 1.785 kilos. Jan. 7, 1924, first blood pressure readings; weight 2.065 kilos.

Feb. 26, feeding experiment began; one egg yolk mixed with powdered "Matzos," daily; weight 2.495 kilos. Apr. 3rd and 4th weeks, rut. Apr. 26, best weight 2.820 kilos. Apr. 30, egg yolk given by stomach tube.

May 2, corneal arc is well formed in right eye, spreading toward center of cornea for a distance of 3 mm. from limbus. In left eye there begins to appear a faint, delicate, white line next to iridocorneal junction. June 28, weight 2.340 kilos.

July 14, two egg yolks by stomach tube, daily. Aug. 19, weight 2.165 kilos.

Aug. 21, animal is cold, wabbly, looks sick. Egg feeding is discontinued. Aug. 22, very weak, cold. Died at 11.30 p.m.

Total number of egg yolks consumed, 210 (without interruption, except isolated days).

Blood Pressure.—Range of figures where highest values were obtained, (*a*) before egg yolk feeding, (*b*) during egg yolk feeding, together with mean of set and pulse rate:

Date	Oscillation	Mean	No. readings	Pulse rate
1924	*mm. Hg*	*mm. Hg*		
(*a*) Jan. 14	128–134	130.4	10	168
Jan. 15	129–134	130.4	10	160
Feb. 26	128–140	133.7	10	
(*b*) Mar. 6	124–139	131.4	20	176
Mar. 27	105–135	115.2	20	160
Apr. 10	118–138	128.6	20	216
Apr. 29	122–143	136.1	19	216
May 3	114–142	134.2	20	216
May 6	120–141	130.9	20	200

Toward the end of May, during the whole of June and first half of July, the pressure reached the lowest values observed during the entire experiment, oscillating between 72 and 108 mm. Hg. Pressure above 120 was recorded on May 17, and the next pressure above 120 was recorded on July 18. The lowest temperature of the room where the measurements were done, was, in this interval of time, 19°C. (May 22, 11.05 a.m.), blood pressure 86–109, mean 97.8 (20 readings), pulse rate 152 per minute. The highest temperature in the same interval, 30°C. (June 24, 3.53 p.m.), blood pressure 85–93, mean 90.1 (10 readings), pulse rate 184 per minute. The thermometer is mounted on the stand of the manometer and the temperature read systematically at the end of the measurements. From July 18 on, the pressure was not as low as in the interval just discussed but reached levels in general not as high as those recorded before May 17, the only exception occurring on August 9, when the pressure rose to 141 after the rabbit moved during the measurement. The fastest pulse rate was 232 per minute, on February 19 and March 12, which did not coincide with the highest pressures. The lowest rate was 128 per minute, on June 28, with a blood pressure between 80 and 86, mean 83.1 (10 readings), and on August 22, when the rabbit was profoundly asthenic and the blood pressure was between 82 mm. and 90 mm. Hg (measured while animal was lying on its side).

Autopsy.—Marked dilatation of heart. Marked atherosis of aorta at root, arch and first portion of thoracic aorta, where intima is thoroughly infiltrated; from here on infiltration is patchy, mainly at the opening of branches, with a rather large patch at the opening of celiac trunk. The infiltration is greatest just beyond the opening of branches, in many instances forming like a crescent on the caudal side of the opening. Lumbar aorta is practically free; iliacs free. Carotids not involved, except at their opening, and excepting a small portion of the root of left carotid (that within loop), in continuation with the aortic infiltration. Aortic leaflets and large mitral cusp are slightly infiltrated. Pulmonary shows a large patch at bifurcation, extending both ways for a short distance. Root of pulmonary is completely free. Profound infiltration of liver, which feels hard, and in many areas is coarse, the capsule in these places being thick and opaque. Spleen large and pale. Adrenals are large; weighed on March 26, 1927

(after almost 3 years in formaldehyde solution), left 550 mg., right 500 mg. Ovaries thoroughly infiltrated, but scarcely larger than normal. Gall bladder much distended, walls not thickened. Bile is very thick. Lungs contain many whitish nodules throughout, not unlike a miliary tuberculosis, and small areas of congestion at both bases. The microscope revealed the presence of a radiating fungus in these nodules.

No. 47-0.—Male, gray, Belgian rabbit. July 7, 1923, left carotid loop is made; weight 2 kilos. July 31, first blood pressure readings. Jan. 12, 1924, weight 2.895 kilos.

Jan. 16, feeding experiment started; one egg yolk mixed with powdered "Matzos," daily. Feb. 16, best weight 3.360 kilos.

Apr. 30, well developed corneal arcs on upper segment of both corneæ. Egg yolk by stomach tube. May 2, corneal arcs spread to lower segments, equatorial parts remaining free. May 11, weight 2.645 kilos. May 12, looks sick. May 15, dead.

Total number of egg yolks consumed, 117 (without interruption).

Concerning the *blood pressure*, see Fig. 1 and discussion.

Autopsy.—Aorta thickened and opaque, intima greatly thickened, yellowish, rough and totally infiltrated from arch down to bifurcation; only spot free from atherosis is the root of the aorta where there is only a small elevated nodule. Pulmonary artery is almost as much involved as the aorta. Root of carotids and iliacs infiltrated. Small amount of fluid in abdomen. Walls of descending and transverse colon are yellowish. Spleen much enlarged, rounded edges, tough. Liver very pale, mottled with yellow. Adrenals very large (left 1.383 gm.; right 1.169 gm. weighed fresh), float in formol solution (10 per cent). Kidneys of normal size and consistency, surface smooth, yellow striations in pyramid following the normal rays; here and there, at base of pyramid, small yellowish nodules. Bilateral purulent pleurisy. Discrete patches of consolidation in left lung. Frozen sections of aorta show the intima to be *twice* as thick as the muscular coat. Histological details are omitted because they add nothing to the present knowledge of this experimental condition.

D 12.—Female, white rabbit. Dec. 10, 1923, left carotid loop is made; weight 1.855 kilos. Jan. 7, 1924, first blood pressure readings; weight 1.970 kilos.

Feb. 26, egg yolk feeding is started; weight 2.435 kilos (one egg yolk in bread, daily).

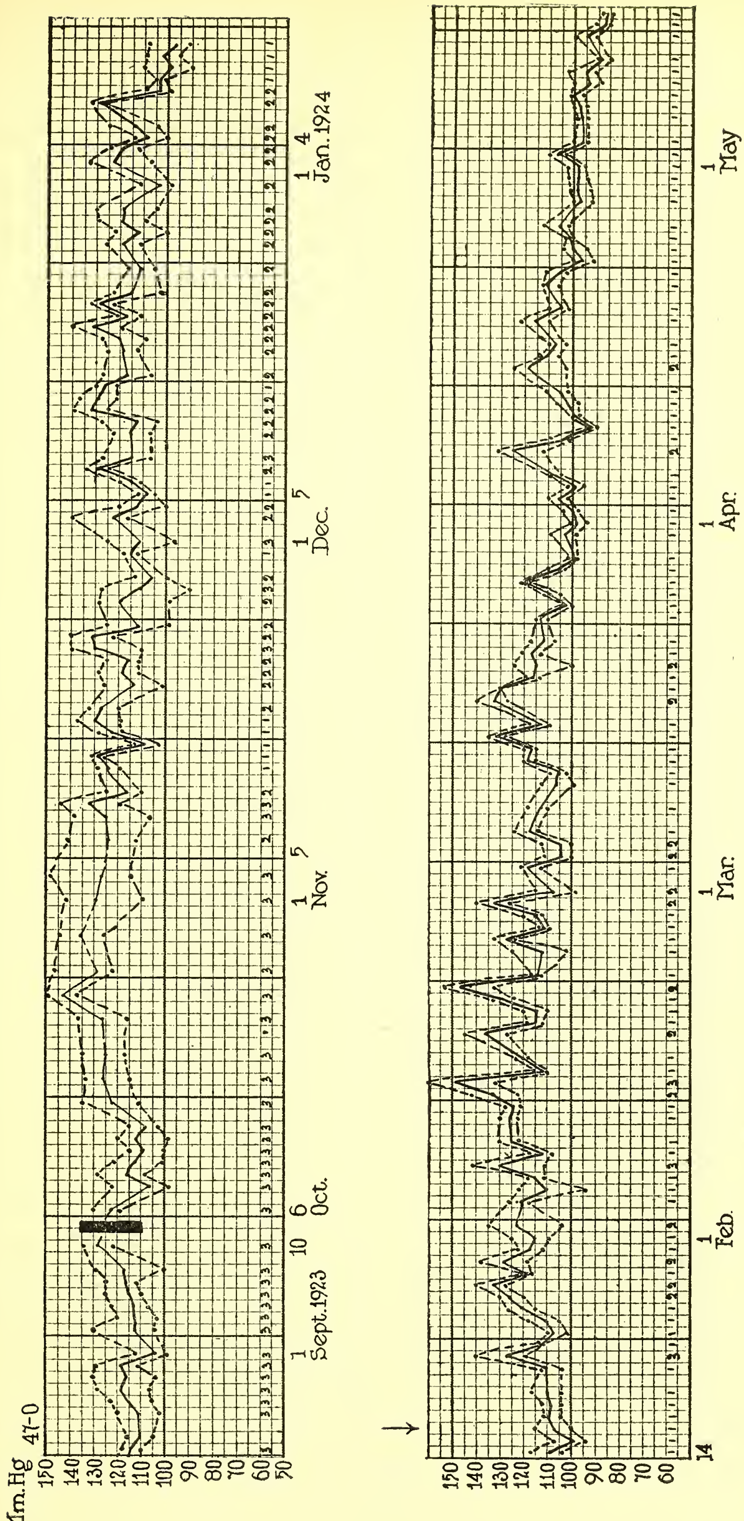

Mm.Hg
47-0
150
140
130
120
110
100
90
80
70
60
50
1
Sept.1923
10
6
Oct.
1
Nov.
5
1
Dec.
5
1
4
Jan.1924
14
1
Feb.
1
Mar.
1
Apr.
1
May

FIG. 1.

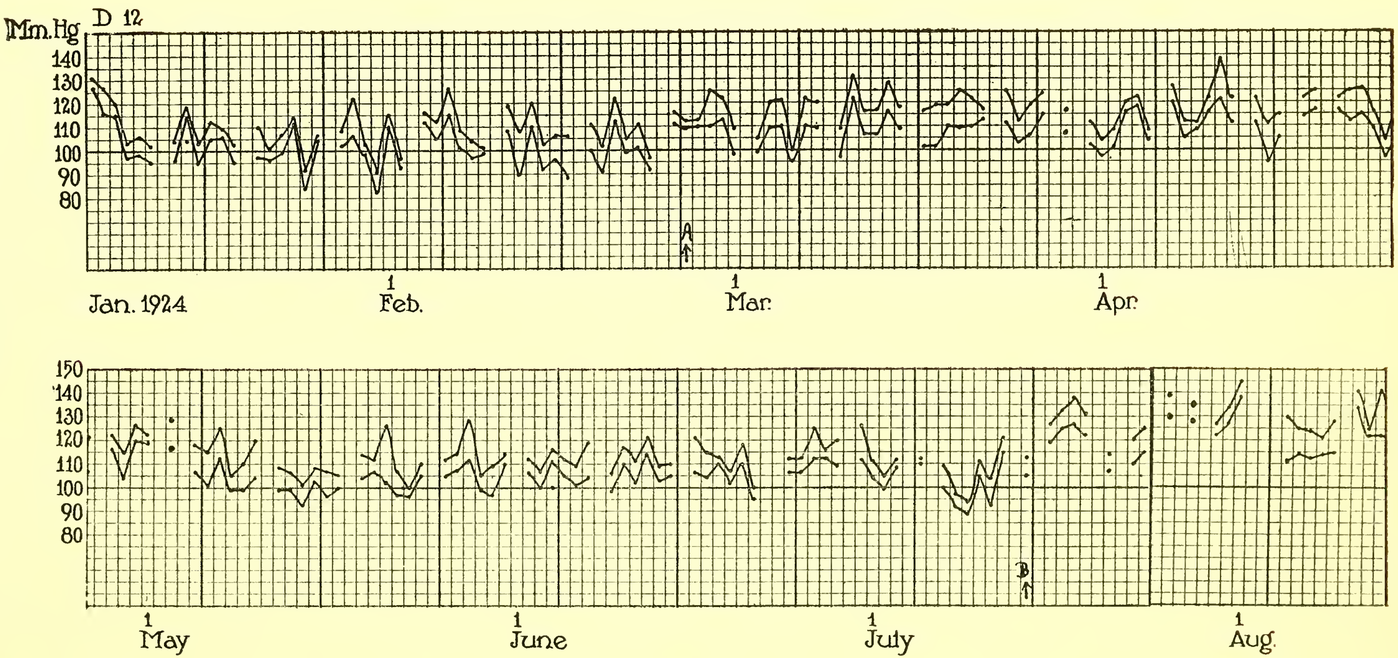
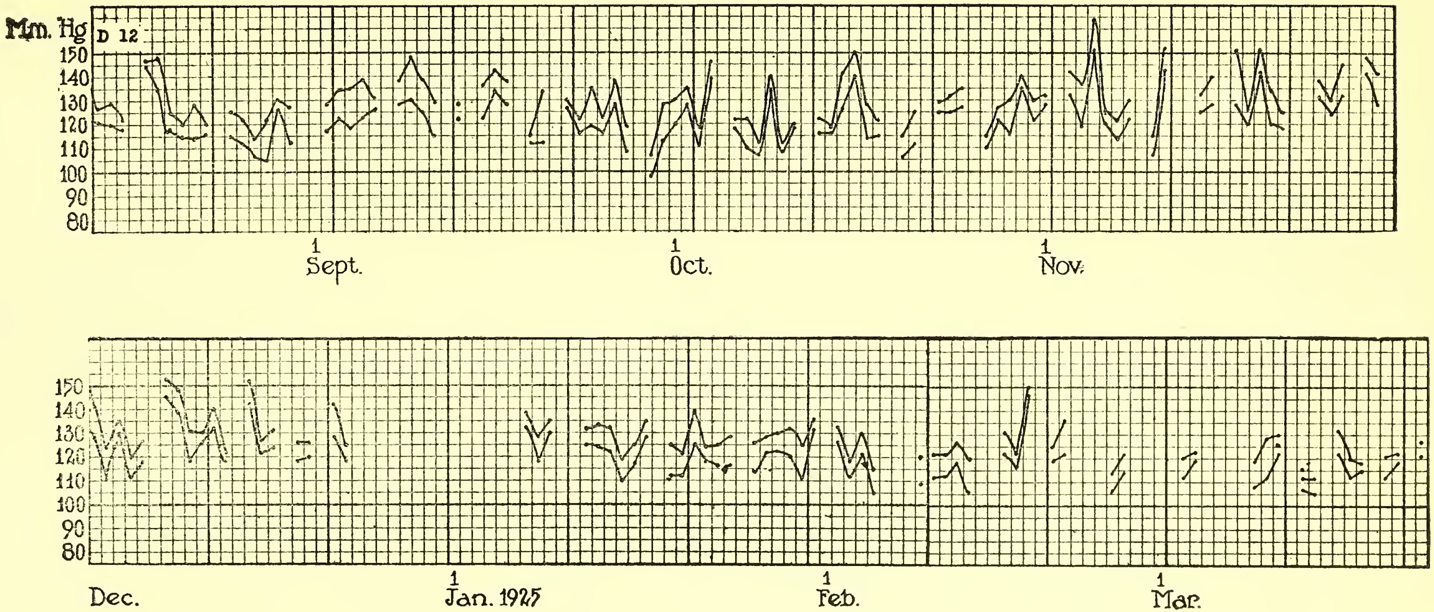

Mm.Hg
D 12
140
130
120
110
100
90
80
A
Jan. 1924
1
Feb.
1
Mar.
1
Apr.
150
140
130
120
110
100
90
80
B
1
May
1
June
1
July
1
Aug.

Fig. 2.



Mm. Hg
D 12
150
140
130
120
110
100
90
80
1 Sept.
1 Oct.
1 Nov.
150
140
130
120
110
100
90
80
Dec.
1 Jan. 1925
1 Feb.
1 Mar.

FIG. 3.

Apr. 30, egg yolk in milk, through stomach tube. May 1, egg yolk in water, stomach tube, daily. Weight 2.615 kilos.

July 14, two egg yolks in water given, daily. Dec. 27, weight 3.335 kilos. Mar. 17, 1925, weight 3.220 kilos.

Mar. 23, animal in excellent condition. It was sacrificed (chloroform) because the experiment was considered to have lasted sufficiently long. The animal did not receive any eggs from Dec. 28, 1924, to Jan. 5, 1925, inclusive. Occasional days scattered throughout the experiment were missed also.

Total number of egg yolks consumed, 531.

For *blood pressure* see discussion. Graph is reproduced in Figs. 2 and 3.

Autopsy.— Aorta and *pulmonary* artery are opaque, thick. Intima is rough, yellowish, wrinkled. In the pulmonary artery the infiltration is greatest around the bifurcation and extends into its branches. In the aorta the infiltration begins at the ring and extends clear down to the iliacs. All the main trunks are infiltrated a short distance from their root. The arch is dilated, and here the infiltration is greatest. Carotids and iliacs are not infiltrated. Mitral valve is thoroughly infiltrated; tricuspid only slightly. Liver: intense fatty infiltration. Costal margin has made a deep impression on convex surface and in this area the infiltration is greatest. Spleen somewhat enlarged. Transverse colon possesses a distinct yellowish discoloration of the mucosa. Uteri are enlarged, and vagina is greatly dilated (contains abundant mucus); cervices, full of clear, transparent polypi. Uterine mucosa is full of like polypi. Adrenals are large, left weighs 570 mg., right 471 mg., weighed fresh. Kidneys normal in size and shape; surface moderately pitted; cut surface shows a few well defined, large yellow nodules in the boundary zone, somewhat spindle-shaped, with the long axis parallel to the medullary rays; besides these large fatty deposits there are fine yellowish striæ in the medulla.

DISCUSSION.

Autopsy Findings.—All the animals developed varying degrees of atherosclerosis of the aorta; from a few flat clusters scattered here and there (D 1) to a total infiltration from root to bifurcation (No. 47-0 and D 12). The most severe lesion was found in No. 47-0 (117 egg yolks) where the fatty intima formed two-thirds of the total thickness of the aorta (7). D 6, after 210 egg yolks, and D 12, after 531 yolks, had a

less profound infiltration, although it extended to the whole length of the aorta in D 12. The least infiltration occurred in D 1, which received the smallest number of eggs (40 eggs). The pulmonary artery was involved in all, the most extensive infiltration occurring again in No. 47-0.

The largest corneal infiltration was seen in D 6, a brown female rabbit. Corneal arcs were absent in D 10 (55 eggs) and D 12 (531 eggs). The former was a brown female rabbit, the latter an albino, female also. Schönheimer (8) concludes, from his experiments, that males are more resistant than females as far as the production of corneal arcs is concerned, and that extensive arcs are obtained only in females after prolonged administration of cholesterol. In accordance with this, the most extensive corneal infiltration was present in a female, but another female had no corneal arcs after 531 eggs. Since this animal was the only albino of the group, and Schönheimer says nothing about the color of the rabbits, this observation should be emphasized. On the other hand, D 10 had no corneal arcs after 55 egg yolks, while D 1, a brown male, had good corneal arcs after 40 eggs.

The fatty infiltration of the liver was most intense in D 12, without any evidence of a cirrhotic process; but in D 6 the liver was firm and in some places distinctly coarse. The spleen was considerably enlarged only in one (No. 47-0). The kidneys showed fatty streaks in the pyramid in three (D 10, No. 47-0, D 12) and discrete clumps in the boundary zone (Bailey) in two (No. 47-0 and D 12).

The adrenals were of good size in all. Unfortunately some of the adrenals were not weighed fresh, but after several years standing in formaldehyde solution. To get an approximate idea as to the effect of such prolonged fixation on the weight, the adrenals of No. 47-0 and D 12, which had been weighed at the time of the autopsy, were weighed again, that is, after an almost equally long formol fixation, with the result that the left gland of No. 47-0 *gained* about 8 per cent (the right had been sectioned and a piece removed for microscopic examination) whereas the adrenals of D 12 *lost* about 4 per cent. If it be assumed that all the other adrenals *gained* 8 per cent (to assume the worst case) and this amount be subtracted from the recorded weight, an average of 667 mg. for the left and somewhat higher for the right is obtained,

far greater than the corresponding averages 238 and 221 mg. for fourteen normal rabbits above 2 kilos in body weight and greater than the averages 353 and 341 mg. for fourteen rabbits above 2 kilos in body weight, whose thyroid and parathyroids had been removed by Marine (Stewart and Rogoff (9)). In the extensive statistical work of Brown, Pearce and Van Allen (10) on 645 normal *male* rabbits, mainly from eastern Pennsylvania and the immediate vicinity of New York City, the mean combined weight of the adrenals is given as 0.383 gm. More details are unnecessary, since all these findings have been described before (8). My sole purpose is to show that the present animals actually had the now well recognized picture of experimental cholesteatosis, and that in particular the aorta showed from slight to extreme fatty intimal deposits. None of these aortas showed any gross calcification of the media. Schönheimer observed a marked calcification of the thoracic aorta in one of his animals, and says, very naively, that it is probably not due to the diet, but rather to the use of the stomach tube, which through repeated, short elevations of the blood pressure, may act like adrenalin injections. Although he had Schmidtmann's method (11) at hand, he did not use it to see if there was really an elevation of blood pressure, which if found, could still be ascribed to struggle or excitement, since the animals do not take the tube without resisting, and granting, of course, that adrenalin necrosis is mechanically produced and not due to a toxic action or something else.

The kidney deserves special mention. Bailey has found it more frequently affected than other investigators (Schönheimer). Bailey found the surface pitted in four out of nine animals egg-fed, or in six out of eight whose kidney showed gross cholesterol lesions. I find striæ and nodules also, but scars in the cortex only in one (D 12), a moderate scarring in fact, and although this animal received nine times as many eggs in less than five times as many days as Bailey's Rabbit 7, the xanthomatose lesions appear insignificant when compared with the extraordinary lesions illustrated in Bailey's paper (his Fig. 7, kidney of Rabbit 7). There were no scars in the kidneys of D1 and D10 which received lead carbonate. I am inclined to believe that these xanthomatose formations are secondary to a preexisting scarring of the cortex, a view considered by Bailey himself and by Schönheimer.

Blood Pressure.—Fig. 1 contains *all* the essential data from No. 47-0. The lower broken line represents the lowest reading of the corresponding day, the upper broken line the highest reading. The solid line is the calculated arithmetic mean. No figure has been discarded, but the measurements taken from July 31 to August 23 have been omitted to shorten the graph. The range of pressure in the omitted period covers from 90 mm. to 134 mm. Hg, with a mean of 110.0 (420 readings). The small figures at bottom of the graph multiplied by 10 give the number of readings of each day. No readings were taken from September 11 to October 5 (black bar in graph), or on isolated days (break in sequence of bottom figures). Egg feeding was started on January 16 (arrow in graph). The only difference in the blood pressure curve before and during the egg feeding consists in the wider range of daily oscillations in the former, a difference readily accounted for by the larger number of daily readings. Toward the end, when the animal was obviously sick (see protocol), the blood pressure was low. The blood pressure reached about 150 on two occasions, both before and during egg feeding. The details of these days are as follows:

Oct. 24, 1923, 10.41 a.m. 145–144–146–144–147–143–144–142–141–141 (pulse rate 200).
10.49 a.m. 149–145–143–144–141–143–145–142–143–146 (pulse rate 160).
10.58 a.m. 144–146–142–142–139–137–137–140–139–138 (pulse rate 160).
Nov. 3, 1923, 3.45 p.m. 129–127–126–126 = 127–126–130–130 = 130–130 (pulse rate 200).
3.51 p.m. 146–148–146 = †128–120–115–116 = 116–116–116 (pulse rate 184).
At the point indicated by † animal moved backward, in box.
3.58 p.m. 129–130–129–128 = 121–120–123–125 = 121–123 (pulse rate 176).
Feb. 14, 1924, 12.08 p.m. 160–160–155–153 = 156–150–152–150 = 150–148–147–152 = 152–147–146–142 = 149–152–150–145 (pulse rate 200). Mouth piece removed for a few seconds, cuff in place, animal did not move.
12.17 p.m. 145–142–135–132 = 139–140–149–140 = 144–146 (pulse rate 188).
4.59 p.m. 118–121–122–122 = 128–129–129–131 = 136–134 (pulse rate 184).

5.07 p.m. 155–153–152–150 = 145–144–144–142 = 143–144 (pulse rate 180).

Feb. 22, 1924, 3.18 p.m. 138–133–139–139 = 142–142–145–143 = 150–150–149–144 = 149–150–150–149 = 150–153–153 = 153 (pulse rate 176).

Pulse rate behaved as follows:

Fastest, Aug. 30, 1923, 248 per minute, with a blood pressure between 125 and 130 mm. Hg, mean 126.7 (first 10 readings).

Slowest, Nov. 27, 1923, 128 per minute, blood pressure 90–104, mean 95.3 (1st 10 readings).

Apr. 19, 1924, 128 per minute, blood pressure 102–110, mean 104.8 (10 readings).

Apr. 30, 120 per minute, blood pressure 94–102, mean 98.3 (10 readings).

May 1, 128 per minute, blood pressure 95–102, mean 98.2 (10 readings).

On May 12, 13 and 14, when the animal was suffering from a pleuropneumonia, the pulse rate was 192, 224, 192, respectively, and the blood pressure oscillated between 84 and 100 mm. Hg. The curve illustrates one of those rare animals which cover the whole range from 90 to 150 mm. Hg, that is, almost the totality of the normal fluctuations of blood pressure in the rabbit. Without special indication it would be impossible to pick out the egg feeding period. D 1 with a maximum pressure of 135 mm. Hg and D 6 with a maximum of 143, may be dismissed without further discussion. D 10 does not differ essentially from No. 47-0.

There remains D 12, whose graph is reproduced in Figs. 2 and 3. During January and February, 1924, up to *A* (see Fig. 2), blood pressure oscillated between 83 and 132 mm. Hg, with a mean of 104.9. From *A* to *B*, 139 days (animal receiving one egg yolk daily), the blood pressure oscillated between 88 and 138 mm. Hg, with a mean of 109.4, the oscillations being somewhat greater during March and April than during May and June. From *B* on to the end of the experiment (animal receiving two egg yolks daily), the blood pressure was in general higher and the oscillations became larger, between 98 and 165 mm. Hg, with a mean of 124.0. Pulse rate behaved as follows:

Fastest, Nov. 5, 1924 (resistance), 256 per minute, blood pressure 151–164 mm. Hg, mean 155 (20 readings).

Nov. 17, 256 per minute, blood pressure 128–151, mean 135.3 (20 readings).

Slowest, June 3, 1924, 132 per minute, slightly arythmic, blood pressure 100–107 mm. Hg, mean 102.1 (10 readings).

July 9, 1924, 136 per minute, blood pressure 89–94, mean 91.4 (10 readings).

The protocols of the days of highest blood pressure are as follows:

Nov. 5, 1924, resistance. 2.32 p.m. 164–164–162 = 160–158–157–155 = 154–154–153–151 = 152–152–151–151 = 153–152–153–153 = 152 (pulse rate 256).

Dec. 1, 1924, 10.25 a.m. 165–163–160–155 = 152–153–149–150 = 145–142–142–142 = 142–142–141–139 = 138–140–139–135 (pulse rate 200).

The curve from *B* on to the end is representative of the average blood pressure of a good number of the normal rabbits. It compares well also with the curve of D 46 (Fig. 6, in a previous paper (5)) before and after double adrenalectomy, but oscillates about a lower level than D 65 (Fig. 4, in the same paper (5)), also adrenalectomized. Taken alone, this part of the curve has, therefore, little interest. It gains interest, only when brought into relation with the first part of the curve, and particularly when it is remembered that from *B* on two eggs were given daily instead of one. But, interesting as it may be, is it significant? The answer may be split in two according to the meaning to be attached to the word "significant." If by "significant" is understood "fluctuations beyond the range of blood pressure of normal rabbits in general" the answer is immediate and negative, as appears clearly enough from my former work (3, 4) and from the preceding paragraphs, or from another glance at the normal curve of No. 47-0 (Fig. 1). If by "significant" is meant "fluctuations within the normal range, but high relatively to the fluctuations of the pressure in the same animal during a period of observation, more or less short, before the experiment," (and these constitute the large majority of the claims found in the literature), then the answer is very difficult, perhaps impossible. It was stated at the beginning of this article that there is a fallacy in ascribing any rise in blood pressure to the experiment which is being done, because sometimes rises of pressure

without known cause were seen in animals employed for observation for a long time. Moreover, D 12 is the only one of five animals in which such an effect is observed. From A to B the animal consumed 130 egg yolks and it is highly probable, judging from the other four experiments and from Bailey's experience, that at the end of that time there was already a good infiltration of the aorta. So that from the standpoint of the etiological relation of high blood pressure to the development of atherosclerosis, this observation is not a favorable one, and the other four are no better. Although the pulmonary artery is just as frequently and almost as severely involved as the aorta, I have not seen any report claiming an increased blood pressure in the pulmonary artery of these rabbits. Not knowing how to measure this pressure, I shall not discuss it, but clearly an examination of this fact will have to be considered by those who believe in the mechanical theory of atherosclerosis and especially by those who may find in experimental conditions material for their clinicopathological speculations. Whatever it may be, these experiments, positive as far as the production of atherosclerosis of the aorta is concerned, are far from satisfying the criterion I have given for a pathologic high blood pressure in rabbits (4).

Analysis of Van Leersum's Work (*2*).—Van Leersum does not mention the number of animals examined, or the length of time during which they were examined. He contents himself with saying that with his method of measuring the blood pressure (an excellent method, I believe) he has determined "regularly and for a very long time" the blood pressure oscillations of normal rabbits and of rabbits subjected to liver feeding. But there is no explicit mention of the actual oscillations found.

He says that all values were recorded, but the first ones usually were not taken into account; that only a series of 5 values which did not differ very much from one another were considered admissible (page 416), but this procedure is arbitrary and Van Leersum does not justify it. The criterion which guided him is as follows: "Die im Anfang erhaltenen Werthe jedoch sind wegen der Unruhe des Thieres in der Regel weniger gelichmässig: der Blutdruck ist dann oft bedeutend erhöht oder ermässigt. Während des Messens kommen die Thiere aber allmählich zur Ruhe und wird der Blutdruck gleichmässiger." For instance, on page 417, where a sample of a protocol is given, 11 figures (9 a.m.) are discarded, from 182 to 205 cm. water, and 5 retained, from 195 to 199 (average 196); at 2 p.m., 5 figures are discarded, from 163 to 170, and the average of the retained figures is 159. It results from all this, that Van Leersum's curves do not repre-

sent the fluctuations of blood pressure of his animals, but are constructed from sets arbitrarily selected among a wide range of values.

He reports the blood pressure of eight animals to which he gave powdered liver and of four more to which he gave sodium taurocholate or sodium glycocholate.

The summary of his results is as follows: No. 87, "average" blood pressure during normal feeding (1 week), 181.6 cm. water; during liver feeding (another week), 194.5 cm. water. No. 57 was given liver every other week. The values (means) as they appear in the graph (Curve 1) are as follows, normal feeding weeks being placed in parentheses: (163) 158 (152) 164 (156) 156 (144) 164 (162) 173 (159) 162 (156) 158. With the exception of 173, which occurs during the liver feeding, all the others are equal to or less than the first figure 163, which happens during a normal period; for differences of 1 cm. of water (less than 1 mm. of Hg) lie within the experimental error. The instance in which the average blood pressure was 173 is less than the average blood pressure of No. 87 during the control period. Rabbit F, early in October, showed an average of 164 cm. (1 week), and in the 1st week of November, still under normal feeding, 180 cm. In the 1st week of liver feeding the average blood pressure was 173, and in the 4th week 181 cm. Rabbit G, early in October, had an average blood pressure of 162.5 cm.; in the 1st week of November, 169 cm. At this time the liver feeding was started. In the 3rd week of this feeding the pressure averaged 171 cm., and in the 6th week 189 cm. water. Van Leersum adds: "Eine Steigung also von gut 11 pCt." But Rabbit F, just mentioned, jumped from 164 to 180 cm., an increase of 10 per cent, without the help of liver feeding. The other four animals may be considered jointly (A, C, D, 23). The averages during the normal feeding period varied between 141 (D) and 172 (C), the observation lasting 1 week for C, 2 for A and D, and irregularly in July, Sept., and Dec., 1911, for No. 23. The maximum weekly average recorded, *during a diet period*, appears as follows: 206 (A), 219 (C), 216 (D), 198 (23) and *after a liver feeding*, 221 (D), in the week following the cessation of the abnormal nourishment.

Van Leersum discusses these findings in the next 3 pages and then comes to the question of what part of the liver is responsible for this effect on the blood pressure. He tries the bile salts (sodium taurocholate and sodium glycocholate) and says: "ihre Wirkung auf den Circulationsapparat ist eine lähmende und sie vermindern den Blutdruck, wie Versuche an vier Kaninchen mich gelehrt haben, in erheblichem Maasse." The protocols are brief but very instructive. I copy from them the pertinent figures. Blood pressure during control period, average, Rabbit Q, 200 cm. water; Rabbit Z, 221; Rabbit X, 208; Rabbit Y, 220. Blood pressure after about 1 month of bile salt (mixed with the food, carrot), average, Rabbit Q, 168 cm. water; Rabbit Z, 190; Rabbit X, 161; Rabbit Y, 182. In other words, figures like 161, which in the main part of the paper are considered normal, are now interpreted as due to the injurious effect of the bile salts, and figures between 200 and 221 which were interpreted before (Rabbits A, C, D, 28) as due to the effect of liver feeding are now considered

normal. If Van Leersum had examined more normal animals and for a longer time, before any experiment was undertaken, he would have interpreted his results differently. I venture this statement because my figures for normal animals include *all* of Van Leersum's figures. 122 cm. water (Curve 6, Nov. 2) and 239 cm. water (Curve 2, Jan. 10) are the lowest and highest figures, respectively, recorded in the papers under discussion, that is, about 90 mm. and 176 mm. Hg respectively. In Fig. 2, Rabbit 489, Graph IV of a previous paper (3), there may be seen a few instances of pressure about 180 (averages, since Van Leersum's figures are averages also) and in Figs. 3 and 4, Rabbit 483, Graphs I, II, and III, there are several below 90.

The analysis may be summarized thus: (1) Van Leersum's range for the normal blood pressure in the rabbit, as recorded by his method, is confirmed; (2) Van Leersum's conclusion concerning the influence of a liver diet on the blood pressure of the rabbit is not substantiated by his data, since the fluctuations of blood pressure he obtained do not surpass his own recorded figures for normal animals.

Note on the Results Obtained by Other Methods.—The work of Fahr, Schmidtmann and Schönheimer, together with an account of the methods used by these authors to measure the blood pressure, has been summarized by Shapiro and Seecof (12), who in their own experiments used Anderson's method. I have answered their criticism of Van Leersum's method elsewhere (4). A few remarks, however, may not be out of place here, particularly since a few reports have appeared subsequently to Shapiro and Seecof's.

1. Van Eweyk and Schmidtmann (11) state that the blood pressure of a healthy rabbit, as recorded by their method, lies between 90 and 100 mm. of Hg. They do not say how many animals they have examined or for how long a period. By comparing their method with the values obtained with a Cowl-Gad's manometer, they find an agreement between their figures and the minimal pressure of the "blutige" measurement. But they add: "Zunächst lassen wir es dahingestellt sein, ob diese Übereinstimmung gesetzmäzig ist oder nicht." In Schmidtmann's first report (13) (six rabbits) the blood pressure was taken *once weekly*. The lowest figure is 88 (Protocol IV) before the experiment, the highest 132 (Protocol VI) in the 2nd and 3rd weeks of liver feeding. In her second report (14), she speaks of feeding experiments (diets rich in cholesterol) on 67 rabbits: in some the blood pressure effect was negative, in the large majority, however, she states that the pressure rose to 120–140 mm. Hg for weeks and even months. Then the blood pressure fell to normal, whereas the blood cholesterol remained high. She explains this fall by assuming an injurious effect of cholesterol on the heart muscle and the vascular system. It is difficult to interpret these data because it is not at all clear what is being measured with that method. The authors think that their figures agree with the minimal pressure of the aorta, but have left "undecided" (their words) how legitimate this agreement is. On the other hand, *one* measurement *once* a week gives entirely too little information on the blood pressure of the rabbit, to draw positive conclusions therefrom.

Schönheimer (8) says that he has also found an increase in blood pressure (Schmidtmann's method) under the influence of cholesterol feeding, but he gives no data, except that the largest increase was from 80 to 112 mm. Hg.

Anitschkow (15) says he has confirmed Schmidtmann's results, but gives no details, not even mentioning the method used.

Deicke (16) reports his findings on 88 rabbits fed on cholesterol or liver. He used Schmidtmann's method, but there is no statement as to how often or how many times the blood pressure was measured. Judging from his graphs, the blood pressure was taken *once a week*, sometimes more than a week apart, sometimes less. The normal curve reproduced shows fluctuations between 96 and 110 mm. The highest values represented in his graphs are, in mm. Hg, 131 (Curve 5, liver feeding), 132 (Curve 6, after intravenous injection of cholesterol solution), 133 (Curve 3, enteral cholesterol), 142 (Curve 2, enteral cholesterol).

Thölldte (17) states that the blood pressure of the rabbit, taken *twice daily* for months (Schmidtmann's method, cholesterol feeding) shows no increase "beyond the physiological fluctuations." Unfortunately he gives no details or figures, so that it is not known what is meant by physiological fluctuations.

2. Anderson's method (18) consists in the recording of the pressure necessary to obliterate the pulsation of one of the ear arteries of the rabbit. The greatest error comes, according to Anderson, from the changes in the caliber of the vessel, but he says that they can be largely controlled by keeping the ears warm. The blood pressure, in his normal animals, ranges between 75 and 90 mm. Hg. That the conditions laid down for the measurements must be closely adhered to is obvious from the recent report of Behrens (19), who devised a method essentially the same as Anderson's, and says that the values obtained in this fashion are very constant and lie about 40 mm. Hg. He does not mention Anderson's work, but quotes the work of Kuraya (20), who, independently of him, designed the same method of blood pressure measurements and obtained the same values (mean blood pressure in healthy animals 35–50 mm. Hg). Shapiro and Seecof (12) used Anderson's method and concluded that "the systolic blood pressure of the central artery of the rabbit's ear averages between 75 and 90 mm. Hg as reported by Anderson." In their table the figures for the controls are not essentially different, ranging between 77 and 105 mm. Hg. They fed lanolin to rabbits which had been subjected to various surgical operations (splenectomy, thyroidectomy, double adrenalectomy and combinations of double adrenalectomy with splenectomy or thyroidectomy). The number of blood pressure readings was very small: from 2 to 6 during the whole course of the experiments. The conclusion reached was that there is no significant hypertension during the developmental stage of experimental lanolin atherosclerosis in rabbits.

SUMMARY.

Egg yolk was fed to five rabbits provided with a good carotid loop (Van Leersum's method). The blood pressure was measured daily

EFFECT ON THE BLOOD PRESSURE OF THE RABBIT OF ARTERIOSCLEROSIS AND NEPHRITIS CAUSED BY URANIUM

INFLUENCE OF OTHER HEAVY METALS *

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In a preliminary publication,[1] it was stated that a severe arteriosclerosis is produced in the rabbit by certain samples of uranium nitrate. The suggestion was made that an impurity might be present in some samples which might in some way be responsible for those observations. Before the experiments were completed, careful consideration was given to all the possible impurities of commercial uranium preparations, and in order to save time it was decided to try, by direct experiment, the influence of two of them: radium and vanadium—the first, because it is the most obvious impurity of uranium, particularly in old preparations;[2] the second, because the literature on vanadium poisoning is meager.[3]

The idea of an impurity, however, soon became untenable on account of subsequent results with uranium alone, as all four samples used were found to produce severe arteriosclerosis occasionally. The experiments with uranium mixtures were, nevertheless, carried further and are now reported together, since they are not without interest; besides, they constitute a good control of the main part of the investigation. One of the rabbits which developed uranium arteriosclerosis had received lead carbonate by mouth some time before, and although lead had not been administered in the other positive cases, there were some differences (to be emphasized later) which required reexamination of the effect of a previous treatment with lead on uranium intoxication. The blood pressure of half of the animals was measured almost daily, before the intoxication and during the whole length of the experiment.

This, then, is a report of the observations on the pathologic processes and the blood pressure in rabbits subjected to intoxication caused by uranium alone and in association with lead, radium and vanadium.

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* From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University.

1. Dominguez, R.: Science **64**:407, 1926.

2. Boltwood, B. B.: Am. J. Sc. **20**:239, 1905.

3. Hamilton, A.: Industrial Poisons in the United States, New York, The Macmillan Company, 1925.

MATERIAL

Sixty-six rabbits were used in these experiments. Carotid loops for the purpose of measuring the blood pressure (Van Leersum) were made in forty-eight (*D,* second column, table 1). Good loops were obtained in thirty-two, and the blood pressure was measured almost daily in all these. Details concerning this method of taking the blood pressure and the results arrived at in the normal rabbit are to be found elsewhere.[4]

Four samples of uranium nitrate were used. These samples were obtained from Eimer and Amend, New York; Merck and Co., Germany; Mallinckrodt Chemical Works, St. Louis, and J. T. Baker Chemical Company, Phillipsburg, N. J., and are referred to in the following as *Ur* 1, *Ur* 2, *Ur* 3 and *Ur* 4, respectively. The surface activity of these samples, tested in an α-ray electroscope, gave ambiguous results, but this method was not considered sufficiently accurate for the purpose.

Radium was purchased from the Radium Chemical Company, Pittsburgh, 1 mg. of radium bromide in 10 cc. of distilled water, and vanadium chloride from Merck and Co., New York.

The uranium nitrate was injected under the skin on the back of the rabbit in concentrations of 1 : 5,000, 2 : 1,000 and 5 : 1,000 in distilled water, once or twice a week. Some animals developed calcified nodules at the site of the injections. Radium was injected subcutaneously in convenient dilutions prepared from the original 10 cc. by means of a pipet calibrated to the hundredth of 1 cc., the amount of radium bromide contained per cc. being calculated as of the order of 1×10^{-5}, 2×10^{-5}, 4×10^{-5} and 1×10^{-4} mg. in the different solutions used. These radium solutions were not all made at once, but were prepared in 50 cc. volumes at a time. In some of these radium solutions, uranium nitrate was dissolved in 2 : 1,000 concentration. From time to time, 0.5 cc. was taken from the bottles (a different pipet being used every time) and allowed to dry on a flat dish or a piece of window glass. The activity of these plates was then tested in an ordinary gold-leaf electroscope, the rate of fall being measured with a stop-watch. The result of one measurement is shown. The solutions were supposed to contain 2 mg. of uranium and 1×10^{-4} mg. of radium per cubic centimeter, and the drying surface, a circle marked out with a wax pencil, was 3.8 cm. in diameter.

Ra (residue of 0.5 cc.) 36.1 divisions per minute

Ur 1 + Ra (residue of 0.5 cc.) 36.4 divisions per minute

Ur 4 + Ra (residue of 0.5 cc.) 45.1 divisions per minute

Ur 1 (residue of 0.5 cc.) 0.67 divisions per minute

Ra standard (1.1×10^{-3} mg. Ra in sealed glass tube 1 mm. thick) 10.5 divisions per minute

Natural leak, 0.25 divisions per minute

Taking into account the natural leak, one sees that the activity of the uranium-radium solution was from eighty to 100 times that of uranium alone. If the plate containing the radium residue was covered with a sheet of writing paper, its activity was reduced to 0.7 divisions per minute. The precise amount of radium in solution was not of interest here. All that was required was to increase the radioactivity of the uranium sample injected and observe the direction of the injurious effect produced by it. A local effect was not observed in the animals which received only radium solutions or radium solutions to which uranium had been added.

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4. Dominguez, R.: J. Metab. Research. **6:**123, 1924; J. Exper. Med. **46:**443 1927.

Vanadium chloride solution, on the other hand, was irritating to the skin in concentrations greater than 1 : 1,000. The fresh solution was blue, but it changed to green in a few minutes. In a few days the vanadium precipitated, so that the solution had to be made fresh immediately before use. The local reaction was the same, whether the blue or the green solution was injected. In the few animals which received uranium and vanadium, each substance was injected separately in a different side.

Lead was given in two forms: lead carbonate smeared on carrots, by mouth, daily, and lead acetate in watery solution (from 1 to 4 per hundred cubic centimeters), by stomach tube, once or twice weekly.

The animals were kept in separate cages, and were fed their usual diet of oats, hay and greens. The body weight was taken every time the injections were made, that is, once or twice every week. After death, the autopsy was carefully performed, special attention being given to the circulatory system and the kidneys. The aorta and pulmonary and main branches were opened and the latter followed as far as possible. A few of the aortas and many of the kidneys were sectioned (frozen section and paraffin embedding) and examined microscopically. In one case (rabbit 17) the brain, heart, thyroid, spleen, liver and kidney were sectioned and examined.

GENERAL RESULTS

The main data of the experiments have been arranged in table 1. The animals of each group have been placed in the order of increasing duration of the experiment, the duration being estimated from the day of the first dose of the toxic material administered to the animal until death. The animals will be referred to by the numbers of the first column.

A few details concerning the way some of the mixed intoxications were conducted will be given next.

Rabbit 17 received lead carbonate by mouth (daily dose gradually increased from 14 to 300 mg.) during the first 180 days. The lead experiment was discontinued because four animals (rabbits 60, 61, 62 and 63) had already come to autopsy and were found to be free from obvious lesions. After ninety-five days, the injections of uranium were started.

Rabbit 18 received 2.40 Gm. of lead acetate in twenty-five days. Four days after the last dose, the injections of uranium were started.

Rabbits 19, 20, 21 and 22 received lead acetate by stomach tube and radium by injection in the first 213, 216, 219 and 216 days, respectively; uranium in the last 96, 120, 120 and 120 days, respectively.

Rabbit 36 received four injections of vanadium in the last thirty days of the experiment.

Rabbit 37 received injections of vanadium during the first 126 days; from then on both vanadium and uranium were administered.

Rabbits 16 and 33 were given uranium alone during the first 235 days of the experiment; uranium and radium were given in the remaining days. The other experiments do not require explanation.

Three rabbits died after one or two injections of uranium (rabbits 2, 38 and 39), and their death was unexplained. Two were given a large initial dose to determine the effect of acute uranium intoxication on the

TABLE 1.—*Summary of Experiments*

Experiment	No.	Sex	Duration of Experiment, Days	No. of Injections of Uranium	Total Amount Administered: Uranium, Mg.	Lead, Gm.	Radium 10^{-4} Mg.	Vanadium, Mg.	Interval Between Last Dose and Death, Days	Weight (Kg.): Initial	Final	Aorta	Pulmonary	Aortic Branches	Kidneys	Blood Pressure Taken	Remarks
Ur 1	1*	M	7	2	10.0	....	....	....	3	3.060	2.800	0	0	0	Swollen	Yes	Acute intoxication
	2*	F	12	2	0.4	....	....	....	4	3.320	.....	0	0	0	....	Yes	
	3	F	76	9	2.14	....	....	....	6	3.460	3.115	++	+	+	....	Yes	
	4	F	88	10	2.32	....	....	....	18	3.355	2.340	++	+	+	Calcified	Yes	
	5	M	133	21	74.0	....	....	....	17	2.680	2.020	++	+	+	Calcified	Yes	Double suprarenalectomized
	6	M	147	13	2.57	....	....	....	4	3.350	2.670	0	0	0	....	Yes	Killed
	7	M	355	40	151.0	....	....	....	47	2.160	1.350	+	0	0	....	Yes	
	8	F	365	48	151.5	....	....	....	10	2.670	1.715	0	0	0	Small	Yes	
	9	F	535	76	196.1	....	....	....	2	2.460	1.745	0	0	0	Small	Yes	
	10	M	572	88	452.5	....	....	....	2	2.340	2.400	0	0	0	....	No	Killed
Ur 1 and Pb acetate	11*	M	57	2	0.4	2.18	....	....	5	2.880	1.975	0	0	0	....	Yes	
Ur 1 and Ra	12*	F	62	5	6.5	....	0.5	....	6	2.315	2.125	+	0	0	Pitted	No	
	13	F	175	19	59.0	....	3.1	....	1	2.265	2.275	+	0	0	....	No	
	14	F	307	33	113.0	....	14.2	....	6	3.705	3.140	0	0	0	....	No	
	15	F	339	42	166.0	....	19.3	....	4	2.185	2.750	0	0	0	....	No	Killed
Ur 1, then Ur 1 and Ra	16	M	428	58	168.0	....	3.9	....	5	2.320	1.665	+	0	+	....	Yes	
Pb carbonate, then Ur 1	17	M	431	9	6.75	11.55	....	....	52	2.225	.....	++	+	+	Calcified	Yes	
Pb acetate, then Ur 1	18	F	84	8	5.0	2.40	....	....	5	3.275	2.885	0	0	+	....	Yes	Killed
Pb acetate and Ra, then Ur 1	19	M	339	16	63.0	4.77	12.7	....	4	2.455	2.500	0	0	0	Pitted	No	Killed
	20	M	343	15	53.8	4.49	13.5	....	8	2.985	2.900	0	0	0	....	Yes	Killed
	21	M	343	22	81.8	5.96	13.5	....	8	3.425	2.840	0	0	0	....	Yes	Killed
	22	F	343	22	83.8	4.68	14.3	....	8	3.470	3.115	+	0	0	....	Yes	Killed
Ur 2	23*	F	6	2	10.0	....	....	....	2	2.650	.....	0	0	0	Swollen	Yes	Acute intoxication
	24	..	53	8	1.8	....	....	....	5	2.215	1.890	+	0	+	Pitted	No	Killed
	25	F	79	14	45.0	....	....	....	3	3.010	2.870	+	0	+	....	Yes	
	26	M	113	20	72.0	....	....	....	4	2.180	2.080	0	0	0	....	No	
	27	F	153	22	7.6	....	....	....	5	2.370	1.890	+	0	+	Granular	No	Meningitis
	28	M	200	26	73.0	....	....	....	15	2.080	1.125	0	0	0	Granular	Yes	
	29	M	344	46	63.8	....	....	....	3	1.950	1.265	+	0	+	Granular	No	
	30	M	355	48	220.0	....	....	....	17	3.280	2.145	0	0	0	....	No	
	31	F	396	63	156.8	....	....	....	2	2.475	1.615	0	0	+	Granular	No	
	32	F	533	76	238.8	....	....	....	12	2.525	1.585	++	0	+	....	No	

Ur 2, then Ur 2 and Ra.......	33	F	579	84	327.0	....	30.9	....	9	2.590	1.985	0	0	0	Small	Yes	Killed
Ur 2 and Ra..................	34	M	339	42	131.2	....	27.3	....	4	2.405	2.660	0	0	0	....	No	Killed
	35	M	343	42	170.0	....	27.7	....	8	2.430	2.560	0	0	0	....	No	Killed
Ur 2, then Ur 2 and Va.......	36	M	495	66	269.0	....	....	20.0	2	2.510	1.780	+	+	0	....	Yes	
Va, then Va and Ur 2........	37	F	233	8	12.0	....	....	74.0	2	2.835	1.740	+	0	0	Granular	Yes	
Ur 3.........................	38*	M	5	1	0.5	....	....	....	5	2.025	.....	0	0	0	....	No	
	39*	F	6	1	0.2	....	....	....	6	1.785	.....	+	0	0	Pitted	No	
	40*	M	6	1	0.5	....	....	....	6	2.340	.....	0	0	0	....	No	Killed
	41	..	85	13	3.9	....	....	....	1	2.760	1.850	++	+	+	....	No	
	42	F	307	47	88.3	....	....	....	9	2.320	1.755	++	0	+	Calcified	No	
	43	F	535	73	223.2	....	....	....	10	2.105	1.420	0	0	0	....	No	
	44	F	535	76	253.8	....	....	....	10	2.240	1.785	+	+	0	Calcified	No	
Ur 4.........................	45	M	190	33	132.5	....	....	....	5	2.485	2.750	0	0	0	Swollen	No	
	46	F	353	50	206.0	....	....	....	5	2.330	1.860	++	+	+	Calcified	Yes	
	47	F	572	88	451.5	....	....	....	2	2.325	2.575	+	0	0	....	No	Killed
Ur 4 and Ra..................	48	F	188	18	57.5	....	4.6	....	8	3.575	3.045	0	0	0	....	No	Killed
	49	M	315	31	97.0	....	11.7	....	21	2.575	1.230	++	0	0	....	No	
	50	F	337	47	190.0	....	51.4	....	2	2.920	2.030	0	0	0	....	Yes	Killed
	51	F	338	42	156.0	....	33.9	....	3	2.175	2.035	0	0	0	Granular	No	Killed
	52	F	338	42	167.0	....	38.95	....	3	2.435	3.050	0	0	0	....	No	Killed
Ra...........................	53	F	155	..	.....	....	3.5	....	2	1.775	2.445	+	0	0	....	No	
	54	F	264	..	.....	....	13.7	....	5	3.255	3.490	0	0	0	....	No	
	55	M	341	..	.....	....	30.4	....	6	2.945	3.050	0	0	0	Pitted	No	Killed
	56	F	341	..	.....	....	33.4	....	6	3.110	3.700	0	0	0	....	Yes	Killed
	57	M	344	..	.....	....	35.3	....	9	2.225	2.950	0	0	0	Pitted	Yes	Killed
Ra and lead acetate.........	58	F	188	..	.....	2.53	8.7	....	4	2.255	2.660	+	0	0	....	No	Pneumonia
Lead acetate................	59	F	19	..	.....	1.70	....	....	2	2.550	2.430	+	0	..	....	Yes	
Lead carbonate.............	60	M	56	..	.....	1.45	....	....	2	1.680	1.735	0	0	..	Pitted	Yes	Pneumonia
	61	M	179	..	.....	1.34	....	....	137	2.140	2.555	0	0	..	....	Yes	Killed
	62	M	183	..	.....	11.92	....	....	4	2.550	2.270	0	0	..	Pitted	Yes	Mediastinal abscess
	63	M	202	..	.....	8.30	....	....	22	1.990	1.695	0	0	..	....	Yes	Pneumonia
Va...........................	64	F	235	..	.....	....	....	149.5	4	2.840	3.230	0	0	0	....	No	Killed
	65	F	237	..	.....	....	....	157.0	6	2.565	2.575	0	0	0	....	Yes	Killed
	66	M	237	..	.....	....	....	161.7	6	2.720	2.430	0	0	0	....	No	Killed

* Received less than eight injections of uranium.

blood pressure (rabbits 1 and 23). Two died of an intercurrent pneumonia (rabbits 11 and 12) early in the experiment. Another became paralyzed and was killed (rabbit 40). Although these eight animals do not properly belong in a study of chronic intoxication, they have been retained because they afford a natural control to the lesions found in the other animals. When these eight animals are excluded (number marked with an asterisk in table 1), forty-four remain which were subjected to uranium intoxication, of which twenty-seven died naturally (whether as a result of the intoxication or of an intercurrent disease) and seventeen were killed. The duration of the first twenty-seven experiments varied from 76 (rabbit 3) to 535 days (rabbits 9, 43 and 44), the total amount of uranium given varying from 2.14 (rabbit 3) to 269 mg. (rabbit 36). The duration of the remaining seventeen varied from 54 (rabbit 18) to 579 days (rabbit 33), the amount of uranium varying from 1.8 (rabbit 24) to 452.5 mg. (rabbit 10).

That the animals developed an increased resistance to uranium as the intoxication proceeded, I take as established. For instance, in the last twenty-three days of the experiment, rabbit 10 received 50 mg. of uranium nitrate in seven injections. The only effect noted was a drop in weight from 2.68 to 2.40 Kg. In the last twenty-nine days, rabbit 22 received 44 mg. in seven injections; the weight dropped from 3.26 to 3.11 Kg.

Of the twenty-seven animals that died as a result of the intoxication, eighteen lost more than 500 Gm. (67 per cent); six lost less than 500 Gm.; two gained; in one (the first animal of the series, rabbit 17), the record is incomplete. The greatest loss in weight was exhibited by rabbit 49, 1.345 Kg. Its best weight was the initial weight, 2,575 Kg. Of the seventeen animals killed in the course of the experiment, five lost more than 500 Gm. (29 per cent), the greatest loss in weight being 890 Gm. (rabbit 50); five lost less than 500 Gm., and seven gained. It is impossible to give more details on this question, because the age of the animals was unknown and the experiments have been heterogeneous in character, duration and amount of uranium. But a certain relation has been found between the loss in weight and the presence of other lesions in the body, to which attention will be called later. The loss in weight was of two general types: either the weight began to fall soon after the first injection and continued to fall progressively until death, or the weight oscillated about a certain level for a considerable time and then fell, more or less rapidly, before death. The former type is considered here as having significance. In some instances the animals easily recovered from a fall in weight if the injections were interrupted or the dose of uranium was diminished; in others, however, the weight continued to fall in spite of these measures, and the animals reached a state of profound emaciation (rabbits 7 and 49).

Three animals died under acute respiratory distress, undoubtedly owing to a terminal renal acidosis (rabbits 3, 4 and 5).

Rabbit 37, referred to previously, showed, a few days after the third injection of uranium and vanadium, a spasticity of the hind legs; rather quick recovery followed. The weight dropped from 2.88 to 2.14 Kg. in seven days. After the fourth injection of uranium and vanadium, the right hind leg became paralyzed, but recovered. Although the injections were given at long intervals and in small amounts, the animal lost 1.06 Kg. in eighty-seven days, after six doses of uranium and vanadium. The paralysis reappeared after the seventh injection, and the animal died two days after the eighth injection. There was not any disturbance of the sphincters, and the cord was normal grossly. This is the only observation of its kind I have recorded.

TABLE 2.—*Frequency of Spontaneous Arteriosclerosis in the Rabbit*

Author	No. Rabbits Examined	Positive Cases	Percentage
Hedinger and Loeb: Arch. f. exper. path. u. Pharmakol. **56**: 314, 1907	About 100	0	0
Miles: J. A. M. A. **49**: 1173, 1907	49	17	34.7
Lucien and Parisot: Compt. rend. Soc. de biol. **64**: 917, 1908	200	10	5
Bennecke: Virchows Arch. f. path. Anat. **191**: 208, 1908	400	12	3
Pearce: J. A. M. A. **51**: 1056, 1908	51	3	6
Weinberg: Compt. rend. Soc. de biol. **65**: 561, 1908	562	37	6.6
Hill: Arch. Int. Med. **5**: 22, 1910	210	31	14.8
Levin and Larkin: Proc. Soc. Exper. Biol. and Med. **7**: 109, 1910	240	31	12.9
Ophüls: Proc. Soc. Exper. Biol. and Med. **8**: 75, 1911	50	0	0
Loeb: Arch. f. exper. Path. u. Pharmakol. **69**: 114, 1912-1913	429	0	0
Van Leersum: Virchows Arch. f. path. Anat. **217**: 452, 1914	132	4	3
Saphir: Am. J. Path. **1**: 403, 1925	50	3	6
Goldblatt: Unpublished data	201	31	15.4

Results of the autopsy of the animals which received radium, vanadium or lead, in the doses used, were essentially negative.

The lesions found at autopsy on animals which received uranium alone or combined with other metals will be considered separately later. They involve the aorta, pulmonary artery, aortic branches, kidney, stomach wall and general musculature of the body. But before presenting these observations I shall review the question of spontaneous arteriosclerosis in the rabbit, and at the same time consider the sclerosis produced by intravenous injections of epinephrine hydrochloride.

SPONTANEOUS ARTERIOSCLEROSIS IN RABBITS

The data which I have been able to analyze myself on spontaneous arteriosclerosis in rabbits are presented in table 2.

Other data to which I have not had access are as follows: cases mentioned in Lucien and Parisot's table,[5] without reference to the original; report of experiments on eighteen rabbits by Thévenot

5. Lucien, M., and Parisot, J.: Compt. rend. Soc. de biol. **64**:917, 1908.

(5.5 per cent positive); report of experiments on twenty-five rabbits by Giovanni-Quadri (8 per cent positive); report of experiments on thirty rabbits by Kalamkarov (10 per cent positive), and the report by J. L. Miller [6] of Mironescu's experiments on 300 normal rabbits without evidences of changes. Other data not suited for the table are those of Steinbiss [7] who says, "Ich habe seit dem Begin meiner Versuche 1908 jede Gelegenheit wahrgenommen, nach Spontansklerose beim Kaninchen zu suchen, habe mir ausser den mir bei meinen Material vorkommenden Todesfällen und Tötungen aus andern als dem mit meinen experimenten zusammenhängenden Gründen vielfach Herz und Aorta von Schlachttieren verschafft, so das ich über 500 Beobachtungen an über 3 Monate alten Tieren verfüge." (Since beginning my experiments in 1908, I have taken advantage of every opportunity to look for spontaneous sclerosis in the rabbit, and, in addition to my own animals that have died or have been killed, I have, in a number of instances and for reasons other than those connected with my experiments, procured the heart and aorta of slaughtered animals, so that I now possess 500 observations on animals more than three months old.) It is probable that by "slaughtered animals" Steinbiss means rabbits. Van Leersum [8] and Shapiro [9] interpret it thus. In any case, he finds only two doubtful cases of spontaneous lesions. Brown, Pearce and Van Allen,[10] who found sixteen lesions of the aorta in 350 male rabbits, add in a footnote: "The majority of the lesions of the aorta occurred in the first 100 rabbits and most of them were comparatively slight degenerations. Subsequently only marked lesions were recorded." Their 4.6 per cent does not represent the totality of the gross lesions observed, and the authors do not explain what is meant by "marked lesions."

Clarkson and Newburgh [11] state, after examination of the aortas of 100 rabbits from laboratory stock, that "a certain type of abnormality was not infrequent. This lesion always occurred in the region of the arch. It was uniformly small, being about 1.5 mm. in diameter. It was a very white, slightly raised process with a depression in its center." Finally, there are abundant reports of attempts to produce lesions with a variety of means, in which no lesions were obtained. This material will not be considered here, but if the naturally occurring arterial lesions in

6. Miller, J. L.: Spontaneous Arterial Degeneration in Rabbits, J. A. M. A. **49**:1789 (Nov. 28) 1907.

7. Steinbiss, W.: Virchows Arch. f. path. Anat. **212**:152, 1912.

8. Van Leersum, E. C.: Virchows Arch. f. path. Anat. **217**:452, 1914.

9. Shapiro, S.: J. Exper. Med. **45**:595, 1927.

10. Brown, W. H.; Pearce, L., and Van Allen, C. M.: J. Exper. Med. **42**:163, 1925.

11. Clarkson, S., and Newburgh, L. H.: J. Exper. Med. **43**:595, 1926.

the rabbit were as common as some authors believe, negative experiments would be less frequent than they actually are.

Table 2, as it stands, represents an important amount of data from different countries, extending over twenty years. The best compilation, in my opinion, is Weinberg's, not only on account of the number, but because his 562 rabbits were "destinés à l'alimentation," and, therefore, not subjected to any experimental procedure. His animals were in three lots, 516, 21 and 25, and the percentage of arteriosclerosis was 6, 19 and 4, respectively. In 130 more rabbits, which had been used for different experiments, he found 9 cases of aortic lesions (6.9 per cent). The total would give 692 rabbits, 46 with arteriosclerosis (6.6 per cent). The most important statistics, from my immediate point of view, are Dr. Goldblatt's, since he has worked in the same building with me (department of pathology), and his rabbits came from the immediate vicinity of Cleveland, like mine, although from different dealers. Besides the aorta, he has examined systematically the pulmonary artery and the main aortic branches, obtaining negative results. His search has been invaluable to me. My own cases were in forty-eight rabbits, of which only a few were not experimented on. The others were subjected to double suprarenalectomy or guanidine intoxication, or they died during the preparation of the carotid loop or as the result of an intercurrent disease. Among these forty-eight rabbits, I found seven with arteriosclerosis of the common type (14 per cent), namely, discrete patches in the ascending arch. In one a few patches were also present in the transverse portion of the arch and in the abdominal aorta. Lesions were not found in the pulmonary or aortic branches in any of the animals.

The statistics of Miles stand out on account of the exceptional frequency of the spontaneous lesions. Her work was published at the peak of the enthusiasm created by Josué's discovery,[12] and many thought that it appeared to disprove the reality of arteriosclerosis caused by epinephrine hydrochloride. As time passes, however, and more normal rabbits are examined, her results appear to have been local, which demands explanation as well as confirmation. Now and then, a suggestion is offered. Thus, for instance, à propos of Crawford's work[13] on "loco-weed disease," an editorial in the *Journal* of the American Medical Association[14] reads, "May not the greater frequency of this 'spontaneous' degeneration in Colorado be due to the forage plants of the region containing minute amounts of some mineral poisons (possibly

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12. Josué, O.: Compt. rend. Soc. de biol. **55**:1374, 1903.

13. Crawford, A. C.: United States Department of Agriculture, Bureau of Plant Industry, 1908, Bull. 129.

14. Radium a Cause of Loco-Weed Disease, editorial, J. A. M. A. **51**:1338 (Oct. 17) 1908.

barium among them) taken up from the soil?" But the work of Marsh, Alsberg and Black[15] does not support Crawford's idea, and the suggestion in the editorial just quoted proved negative in the hands of Hill,[16] as far as an injurious effect of barium chloride on the aorta is concerned. Uranium could logically be suggested now, but if I were to suggest anything, it would be to repeat Miles' work and see if the next 49, or better 100, rabbits in Colorado show again a high percentage of arterial lesions.

The nature of the lesions is not as discordant as their frequency. The large majority consists in a few small discrete patches situated in the thoracic aorta, in the arch especially. In a few cases the lesions involved the whole length of the aorta: two in Miles' collection and three in Lucien and Parisot's. One of Weinberg's rabbits had many small calcareous plaques in the thoracic and first portion of the abdominal aorta, accompanied by dilatation of the vessel; and one of Hill's, a young rabbit weighing 940 Gm., had a lesion appearing "as a diffuse roughening of the thoracic and upper abdominal aorta." Pearce[17] has presented, besides the fifty-one rabbits of the table, eleven more which received substances of one kind or another (dog's serum, nephrotoxic serum, human blood, typhoid bacillus, chrome salts), and four of them showed lesions. In two of these rabbits "the lesions were diffuse involving irregularly the entire aorta and comparable to those produced by adrenalin." If I put Pearce's additional 11 rabbits in the table, I obtain out of 183 positive cases 9 in which the lesion was extensive or severe.

The lesion that is found spontaneously and that which is produced by intravenous injection of epinephrine hydrochloride consist essentially in a calcification of the aortic media, like the lesions here reported. It will be of some use then, to summarize briefly the observations in sclerosis from epinephrine hydrochloride. Saltykow's[18] exhaustive review has been of considerable assistance to me, but I have consulted the original whenever the lesion seems to be at all extensive. Cases in which the totality of the aorta was involved are few: one each reported by Pic and Bonnamour,[19] Külbs,[20] Lortat-Jacob and

15. Marsh, D. C.; Alsberg, C. L., and Black, O. F.: United States Department of Agriculture, Bureau of Plant Industry, 1912, Bull. 246.

16. Hill, M. C.: Various Forms of Experimental Arterial Disease in the Rabbit, Arch. Int. Med. **5**:22 (Jan.) 1910.

17. Pearce, R. M.: Occurrence of Spontaneous Arterial Degeneration in the Rabbit, J. A. M. A. **51**:1056 (Sept. 26) 1908.

18. Saltykow, S.: Centralbl. f. allg. Pathol. u. path. Anat. **19**:321 and 369, 1908.

19. Pic. A., and Bonnamour, S.: Compt. rend. Soc. de biol. **57**:219, 1905.

20. Külbs: Arch. f. exper. Path. u. Pharmakol. **53**:140, 1905.

Sabaréanu,[21] Scheidemandel,[22] Loeb and Githens,[23] Bonnamour and Thévenot,[24] except Lortat-Jacob and Sabaréanu [21] who had three or four cases (description incomplete, no illustrations), involving a total of about nine rabbits. The most intense of these cases seems to be the one reported by Scheidemandel, according to whose description the aorta was transformed into a rigid, beaded tube, "welches sich am besten mit den bekannten Bildern einer stark verkalkten, gänsetracheenartigen Femoralarterie des Menschen vergleichen lässt" (which may be best compared with the well known pictures of a markedly calcified femoral artery in man, resembling the trachea of a goose). In this same animal Scheidemandel saw a calcification in rings (ringförmige Mediaverkalkung) in the branches of the pulmonary arteries, but no calcification of the aortic branches, although he describes a thickening of their walls. Lesions in the peripheral arteries are infrequent in sclerosis caused by epinephrine hydrochloride.[18] Of the innumerable experiments reported on arteriosclerosis induced by other means, I shall mention only two. One was reported by Israel [25] (extirpation of one kidney and repeated injections of alcohol in the remaining one), whose description is as follows: "Die Kalkplatten finden sich überwiegend an der vorderer Hälfte im aufsteigenden und unteren Brusttheil der Aorta, an der Hinteren im oberen Brusttheil, während Bogen und Bauchtheil relativ frei sind." (The calcified plaques are found chiefly in the anterior portion of the ascending aorta and the lower thoracic aorta, and in the posterior portion of the upper thoracic aorta, whereas the arch and the abdominal aorta are relatively free.) The description is accompanied by a good illustration. The other case was reported by Hedinger and Loeb; [26] besides the calcified plaques of one of their rabbits (subcutaneous injections of potassium iodide) transverse striations were found in the aorta.

Summary.—Spontaneous arteriosclerosis in the rabbit varies from place to place, being in some localities rare or absent, in others, Colorado in particular, frequent. The mean of more than 3,500 rabbits examined during the last twenty years in this country and in Europe is less than 6 per cent. The proportion of severe cases among normal rabbits is rare (about 4 per cent of the positive cases, less than 0.3 per cent of the total). The pulmonary and peripheral arteries, with the exception of

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21. Lortat-Jacob, L., and Sabaréanu, G.: Compt. rend. Soc. de biol. **58**:583, 1905.

22. Scheidemandel, E.: Virchows Arch. f. path. Anat. **181**:363, 1905.

23. Loeb, L., and Githens, T. S.: Am. J. M. Sc. **130**:658, 1905.

24. Bonnamour, S., and Thévenot, L.: Compt. rend. Soc. de biol. **66**:387, 1909.

25. Israel, O.: Virchows Arch. f. path. Anat. **86**:299, 1881.

26. Hedinger, E., and Loeb, O.: Arch. f. exper. Path. u. Pharmakol. **56**:314, 1907.

the iliacs, are not affected. In the vicinity of Cleveland, the frequency of the lesions during the past three years has been about 15 per cent, all having been minimal or slight (single or conglomerate patches, with and without calcification, at the arch of the aorta).

In regard to sclerosis caused by epinephrine hydrochloride, I shall stress the following points: the cases in which the totality of the aorta is involved have been rare (less than 3 per cent in about 400 reported experiments, approximate figures); the pulmonary and peripheral arteries are rarely affected; aneurysm formation is common, and ring arrangement of the calcified lesions is inconspicuous.

ARTERIOSCLEROSIS CAUSED BY URANIUM

Aorta.—Lesions of all degrees were found in twenty-three of fifty-two animals which received uranium, alone or together with other substances. If eight animals are excluded, because of the small number of the injections of uranium, there remain twenty-one instances of lesions of the aorta, of all degrees, among forty-four rabbits experimented on, 47.7 per cent. Grossly the aortic lesions present all gradations of severity, from minimal lesions to a profound calcification of the whole vessel. Besides the ordinary calcified plaque commonly observed in spontaneous arteriosclerosis and in experiments with epinephrine hydrochloride, some aortas exhibit a striking annular calcification which I regard as new. The lesions have been classified as severe (+ + in table 1) when the totality or almost the totality of the vessel showed calcification, or when the calcification assumed a distinct annular arrangement; and slight (+ in table 1) when the lesions were few in number, and involved one or at most two regions of the aorta. Reference to the table will show that of twenty-one instances of lesions in the aorta (the numbers with an asterisk always being discarded), ten were "severe." A short description of two of them will suffice.

In rabbit 17, the aorta was dilated and rigid. On the external surface were transverse white hard rings, which gave to the vessel the appearance of a trachea. When the aorta was opened, the calcification was found to be an almost continuous process from the root down. The calcified plates and rings of the wall broke on flattening out the aorta. The elasticity was lost, except at the lumbar portion.

In rabbit 41, the whole of the aorta, from the root down, contained confluent rounded plaques involving the entire circumference of the vessel. Practically the entire aorta was unhealthy.

The remaining eight cases were not as severe as the two just described. A roentgenogram of the aorta of rabbit 49 (fig. 3) showed that the rings extended from the root to the opening of the renal arteries. The ascending arch was dilated and totally calcified. The lesions were least severe in rabbit 3, but the nature of the lesions was such (fig. 2)

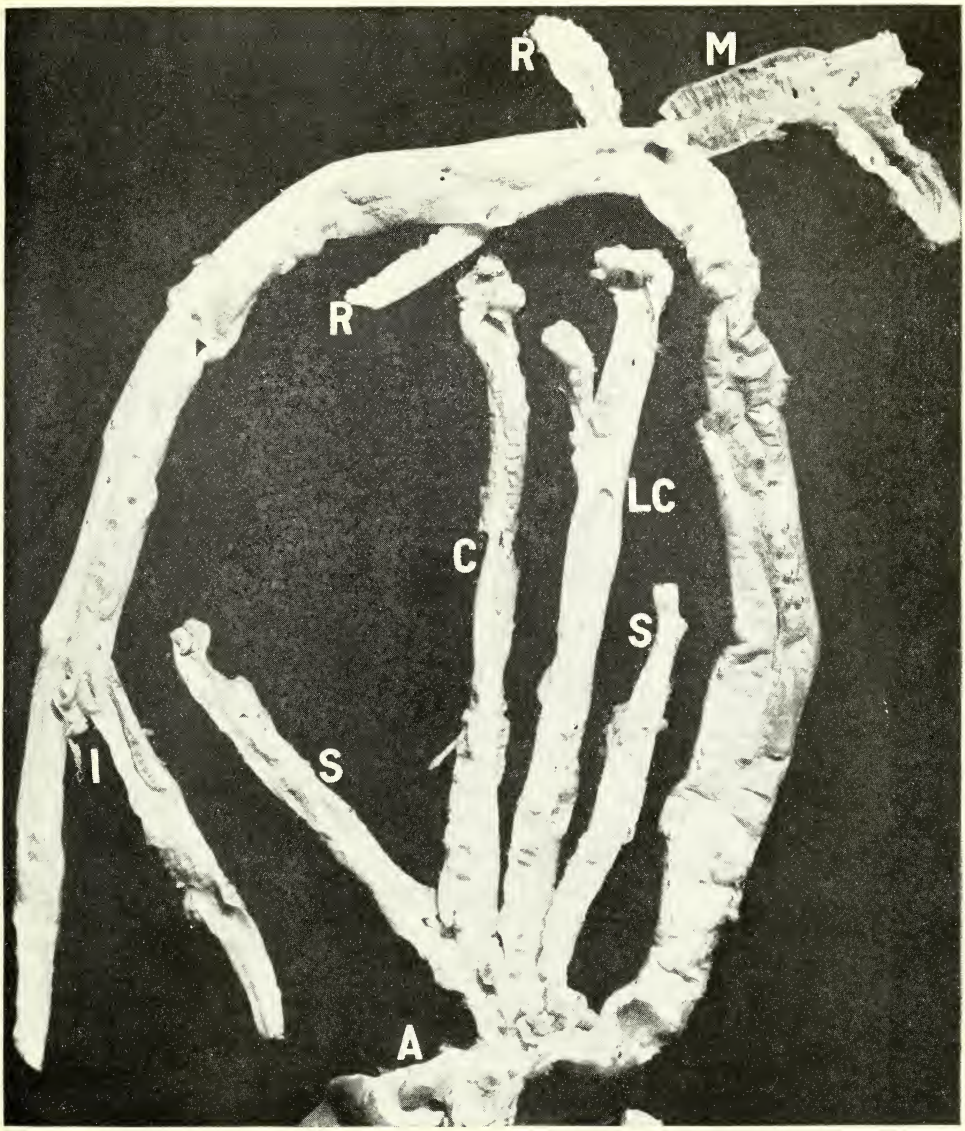

Fig. 1 (rabbit 5).—Aorta and branches; *A* indicates aorta; *C*, carotid; *L C*, left carotid (in the loop during life); *S*, *S*, subclavian ar teries; *M*, superior mesenteric artery; *R*, *R*, renal arteries, and *I*, iliac arteries. The sev ere changes in the mesenteric artery and the rings in the mesenteric iliac and subclavian ar teries should be observed. The discrete lesions of the renal arteries are not seen in the photog raph. The carotid in the loop was less affected than the other one.

that I cannot classify this calcification with anything described before in experimental arteriosclerosis. The thoracic aorta of rabbit 3 was dilated, but free from gross lesions. An approximate idea of the appearance of the aortas in the other cases (rabbits 4, 32, 36, 42 and 46) may be obtained from figure 1 (rabbit 5).

Of the eleven rabbits whose lesions have been called "slight," one (rabbit 27) had a few small plaques at the root and three good-sized plaques at the bifurcation; another (rabbit 37) presented a large plaque

Fig. 2 (rabbit 3).—Lumbar aorta and common iliac arteries. Calcified rings forming hard ridges on intima.

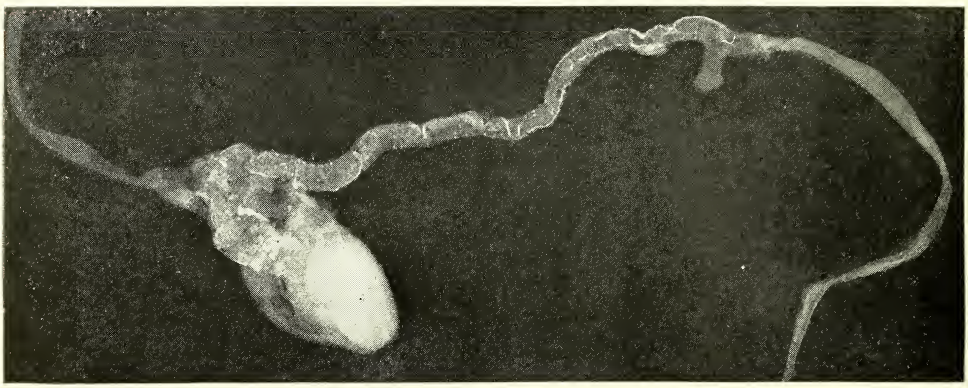

Fig. 3 (rabbit 49).—Roentgenogram (positive). The marked dilatation of the root of the aorta should be noted. Calcified rings are seen as transverse white lines. The process stops at the root of the renal arteries.

on the convexity of the ascending arch, a few small calcified plaques above both coronary cusps and a sharp calcified transverse streak on the ascending arch. The remaining nine (rabbits 7, 13, 16, 22, 24, 25, 29, 44 and 47) exhibited small lesions on the arch, which agreed in extent and description with the so-called spontaneous arteriosclerosis. There has not been any instance of the formation of an aneurysm or of mural thrombosis. Microscopically, the lesions are of only one type:

necrosis and medial calcification, like "spontaneous" sclerosis and sclerosis caused by epinephrine hydrochloride (compare Hesse's [27] observations).

Pulmonary Artery.—Lesions were found in eight animals. In the order of decreasing severity, they were found to be as follows: rabbit 17, several small calcified plaques and two small aneurysms (1 mm. diameter); rabbit 46, many calcified plaques in main trunk; rabbit 3, large saccular aneurysm bulging anteriorly (7 mm. diameter); rabbit 41, three calcified plaques; rabbit 5, two large patches of calcification midway between root and bifurcation; rabbit 44, small plaques at root; rabbits 4 and 36, each, one small patch at bifurcation.

Rings were not present in the pulmonary artery of these animals, but it is noteworthy that two cases of aneurysm should have occurred (rabbits 3 and 17).

Aortic Branches.—The main arteries of the body were affected in fifteen animals. The lesions consisted, as in the case of the aorta, of calcified plaques or calcified rings, and corresponded to a medial calcification resembling Mönckeberg's arteriosclerosis.

I have already stated [28] that the carotid within the loop presents certain lesions (transverse striae) that have been ascribed to the operation itself rather than to the manipulations accompanying the blood pressure measurements. Therefore, in order to avoid ambiguities, all lesions in the carotid loop will be omitted from the present discussion.

The distribution of the lesions in these fifteen animals (exclusive of the loop carotid) is as follows:

Table 3.—*Distribution of Lesions in Main Arteries of Body (Exclusive of the Loop Carotid in Fifteen Rabbits*

Location of Lesions	No of Cases	Rabbit Number
All main aortic branches	2	5, 17
All main aortic branches except renal arteries	1	32
Carotid, subclavian, iliac and femoral arteries	2	3, 46
Carotid, renal, iliac and femoral arteries	1	4
Carotid, subclavian and iliac arteries	1	42
Carotid, renal and iliac arteries	1	41, did not have loop
Carotid artery	5	18, 24, 25, 29, 31
Iliac arteries	1	27
Femoral arteries	1	16

In rabbit 17, the lesions assumed the form of rings and not only were present in all the aortic branches but could be followed down the brachial, the femoral arteries, and even the thyroid and parathyroid arteries. The lesions of rabbit 5 may be seen in figure 1. They were

27. Hesse, M.: Virchows Arch. f. path. Anat. **249**:437, 1924.

28. Dominguez (reference 4, second part).

most pronounced in the mesenteric artery. Rings in the iliac arteries (rabbit 3) may be seen in figure 2. Occasionally, the left carotid artery, in situ, shows striations equal to those exhibited by the carotid in the loop, as may be seen in figure 4, in which the vessel on the left represents the loop carotid. For the sake of completeness I may add that in two animals (rabbits 16 and 27) calcified plaques were found in the loop carotid, in addition to the striae, whereas the other carotid was normal. In two other animals (rabbits 18 and 24), on the other hand, plaques were absent in the loop carotid but present in the other one.

Fig. 4 (rabbit 25).—Carotids. *L* indicates loop carotid. Changes in the other carotid are more marked than is shown in the photograph, but less than those of the loop carotid.

RENAL LESIONS

The lesions in the kidney correspond in the main to those described by Dickson.[29] According to the amount of uranium and the duration of the experiment, various types of anatomic injuries were found, from the large swelling of an acute nephritis (rabbits 1 and 23) to an extraordinary shrinkage of the kidneys with fine granulation of their outer surface (rabbits 27, 28, 29, 31, 37 and 51). The most common

29. Dickson, E. C.: Further Report on Production of Experimental Chronic Nephritis in Animals by Administration of Uranium Nitrate, Arch. Int. Med. **9:** 557 (May) 1912.

lesion found consisted of a reduction of the cortical thickness, with obliteration of the normal striations, fine grayish mottling of the external surface without granulations and an intense congestion of the base of the pyramid. Microscopically, these lesions correspond to a necrotic destruction of the tubules, with and without calcification.

In six animals (rabbits 4, 5, 17, 42, 44 and 46), besides some of the lesions just mentioned, there was a marked calcification, seen with the naked eye as a regular white band at the junction of the outer and middle thirds of the pyramid. On close inspection this band could be resolved into fine streaks along the medullary rays. Microscopically the calcium deposits were found to occur on the limbs of Henle's loops.

Two animals (rabbits 4 and 17) also presented a calcification of the interlobular and afferent arteries, and calcification of the Bowman's

Fig. 5 (rabbit 17).—Photomicrograph of kidney; magnification, × 113.5; frozen section, Van Kossa's stain; *a* indicates artery and *c*, glomerular capsules. Other calcified areas lie in or around tubules.

capsules, to which attention was called previously.[1] These lesions are well illustrated in figures 5, 6 and 7. Rabbit 17 showed, moreover, a marked destruction of the glomeruli, with calcium deposits in the tufts.

GASTRIC LESIONS

The lesions found in the stomach consist in a calcification of the longitudinal muscular coat. Four cases have been recorded (in rabbits 16, 32, 44 and 46), but it must be conceded that, this lesion being entirely unexpected, several cases may have been overlooked before the stomach was systematically examined. The first instance was observed on Oct. 19, 1926 (rabbit 46), and before this day twenty-six of the rabbits in the experiments had already come to autopsy.

The calcification was extensive in three animals (rabbits 32, 44 and 46). A large portion of the anterior and posterior walls of the stomach of these rabbits was covered with white, hard deposits of lime salts, more abundant toward the greater curvature than toward the lesser curvature. These deposits formed streaks and narrow plaques which followed, in a general way, the direction of the longitudinal axis of the stomach. The fundus and pars pylorica were free. In the stomach of rabbit 16 there was only a patch of calcification in the anterior wall of the antrum.

The mucosa was normal in all except rabbit 46. In this animal, the mucosa of the pars pylorica, over the convex border of the organ, and that of the fundus showed several edematous areas, some of which were

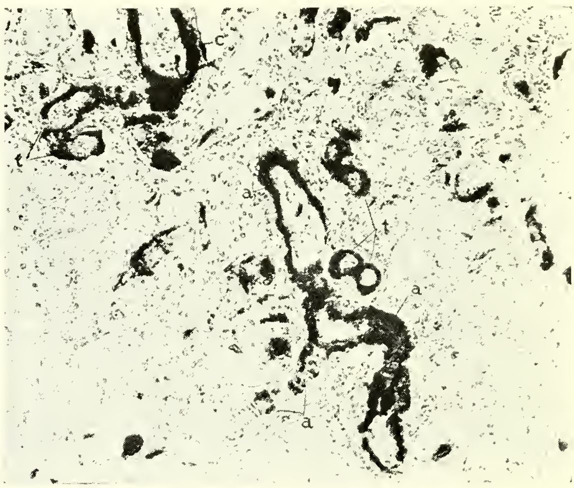

Fig. 6 (rabbit 17).—Another section of kidney; same stain and magnification as in figure 5; *a* indicates artery; *c*, glomerular capsules, and *t*, tubules.

hemorrhagic and were covered with a pseudomembranous exudate. The stomach in this case contained practically no food, but instead about 15 cc. of a milky mucus.

Under the microscope the calcium deposits are found in the outer longitudinal layer of smooth muscle, beneath the serosa. In the immediate neighborhood of these deposits there is fibrosis, hyalinization of connective tissue and a moderate infiltration of mononucleated cells. In decalcified sections the necrosis of smooth muscle is evident. Sections of the hemorrhagic portions of the stomach of rabbit 46 showed the lesion to be in the nature of a hemorrhagic infarction, but an anatomic cause for it was not found. The calcification was localized to the outer muscular coat, and the vessels were intact.

STRIATED MUSCLE

In one animal, rabbit 7, practically all the striated muscles of the body showed calcification. It appeared to the naked eye as long whitish streaks, following the direction of the fibers of the corresponding muscle. The muscles most involved were those of the neck, the serratus major and the abdominal muscles (fig. 8). The diaphragm showed a moderate number of calcified streaks. The muscles of the extremities were the least involved, some appearing normal. Under the microscope (hematoxylin-eosin stain) the lesion appeared as a necrosis of the muscle fiber accompanied by calcification, and with a slight inflammatory reaction. The topography of the muscle was, in general, well preserved. In decalcified sections, the central portion of the lesions had disappeared,

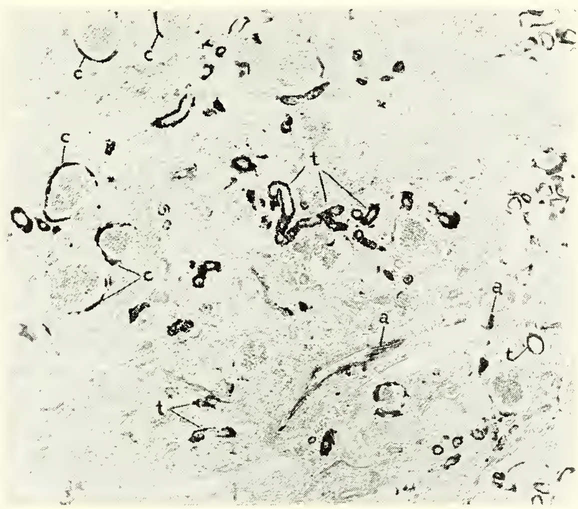

Fig. 7 (rabbit 4).—Photomicrograph of kidney. Frozen section, Van Kossa's stain; *a* indicates artery; *c*, glomerular capsules, and *t*, tubules.

leaving a ragged space bordered by hyaline masses, granular matter and mononucleated cells. At the periphery there was a hyalinized connective tissue with scanty infiltration, in some of the lesions; in others, there was only a slightly thickened connective tissue. As in the case of calcification in the stomach, several of these lesions may have been overlooked before the existence of this muscular injury was recognized. The autopsy of this animal was performed on Oct. 21, 1926, only two days after the first gastric lesion was discovered.

OTHER LESIONS

Osteoporosis has been observed in several animals. It was especially pronounced in rabbits 7 and 44. The ribs of both these animals broke

like cardboard, and the calvarium of the former could be torn away with forceps. Siderosis of the liver was found in several; it was particularly marked in rabbit 36. Finally, there were some of the usual diseases found in rabbits, as coccidial cysts, purulent otitis media and scarred (pitted) kidneys. Some of these are shown in table 1.

COMMENT ON ANATOMIC LESIONS

It is clear from the foregoing experiments that the severe lesions of the aorta cannot be considered as cases of "spontaneous" arteriosclerosis. The "slight" lesions are indistinguishable from the "spon-

Fig. 8 (rabbit 7).—Right side, head to the left, abdomen upward. The white calcified streaks in the serratus major, abdominal muscles and pectoralis minor should be noted.

taneous" ones, but since in "spontaneous" arteriosclerosis lesions do not occur in the pulmonary artery and aortic branches, with the possible exception of the iliac arteries (Miles [30]), it is not unreasonable to consider as positive cases caused by uranium such "slight" aortic injuries as were associated with lesions elsewhere in the circulatory system, and also those in which the main arteries were involved, without aortic injury. Selecting the cases on these grounds I obtained nineteen which

30. Miles, A. B.: Spontaneous Arterial Degeneration in Rabbits, J. A. M. A. **49**:1173 (Oct. 5) 1907.

may be regarded as positive arteriosclerosis caused by uranium. The interrelations of the lesions in these nineteen animals are seen in table 4.

There may be some difficulty in accepting case *7* as one of arteriosclerosis caused by uranium. The lesion in the aorta was slight. There was not any other injury to the circulatory apparatus, but a severe one to the striated musculature of the body. The question remains, was this muscular calcification caused by uranium? I do not know that it was caused by uranium any more than I know that the arterial lesions were caused by it. In the arterial lesions, the severity and the

TABLE 4.—*Interrelations of Lesions in Nineteen Animals with Arteriosclerosis Caused by Uranium*

	Location of Lesion			Calcification in			
Rabbit	Aorta	Pulmonary	Arteries	Kidney	Stomach	Musculature	Uranium Sample
3	++	+	+	..	..	..	1
4	++	+	+	+	..	..	1
5	++	+	+	+	..	..	1
17	++	+	+	+	..	..	1
32	++	0	+	..	+	..	2
36	++	+	0	..	..	..	2
41	++	+	+	..	..	..	3
42	++	0	+	+	..	..	3
46	++	+	+	+	+	..	4
49	++	0	0	..	..	..	4
7	+	0	0	..	..	+	1
16	+	0	+	..	+	..	1
24	+	0	+	..	..	..	2
25	+	0	+	..	..	..	2
27	+	0	+	..	..	..	2
29	+	0	+	..	..	..	2
44	+	+	0	+	+	..	3
18	0	0	+	..	..	..	1
31	0	0	+	..	..	..	2
No. of cases 19	17	8	15	6	4	1	

frequency of the lesions warranted the inference of an intimate relationship between the two. Only one case of injury to the striated muscle was observed, but the objection was weakened by the fact that the musculature of the body was not carefully examined in one half of the rabbits, and only when the lesion was so severe that it could not be missed was it recognized. On the other hand, the lesions had everywhere the same pattern, whether they were in the arterial wall, the loops of Henle, the stomach or the striated muscle, namely, necrosis and calcification. I have, therefore, no reason for eliminating this case from the table.

The differences observed in the character and the distribution of the lesions must be looked for in the complex reaction of the animal organism to injury. The following data taken from table 1 will help to

make clear these variations in response: The severe cases of aortic calcification occurred in ten experiments lasting from 76 to 533 days, in which the number of injections varied from nine to seventy-six, and the amount of uranium nitrate from 2.14 to 269 mg. Rabbits 10 and 47 received 452 ± 0.5 mg. in 572 days and were killed while in apparent good health; their aortas were normal. The gross calcification of the kidney was just as erratic. On the other hand, the calcification in the stomach wall appeared more consistently as a late injury in chronic intoxication caused by uranium. In four positive cases (rabbits 16, 32, 44 and 46) the experiments varied in duration from 353 to 535 days, in the number of injections from fifty to seventy-six and in the amount of uranium from 168 to 253.8 mg. The results were similar for the calcification of striated muscle (355 days, forty injections and 151 mg. of uranium).

The question of loss in body weight may be considered now. Of twenty-seven animals which died of intoxication caused by uranium, seventeen showed arteriosclerosis; that is, practically all the positive cases fall in this group. Of these twenty-seven, eighteen lost more than 500 Gm., and among these eighteen, killed, were eleven with arteriosclerosis (seven severe). Of seventeen animals that were killed, only two showed "slight" lesions in the circulatory apparatus (rabbits 18 and 24). Both lost less than 500 Gm. The differences between these two groups of deaths from uranium and the group of animals which were killed are probably real, since both groups compare well in the duration of the experiment and in the amount of uranium given, so that it cannot be said that arteriosclerosis was uncommon or absent among the killed animals because these rabbits received less uranium or because time was not allowed for results to develop. As a matter of fact, the longest experiments were made and the largest amounts of uranium were found among the killed animals (rabbits 10, 33 and 47). Evidently, some rabbits possess a marked resistance toward uranium. Others, to the contrary, exhibit marked susceptibility to it, and in summary of the preceding paragraphs it may be said that among the rabbits which show in their loss of weight and general behavior their intolerance toward uranium, the chances of finding arteriosclerosis are greatest.

Several complicating factors in the nineteen rabbits retained as having positive arteriosclerosis caused by uranium may be discussed now: 1. Operation on the carotid loop: As table 1 shows, in all these nineteen rabbits but one (rabbit 41), a carotid loop had been made. But eighteen other rabbits with carotid loop received uranium and did not show arterial lesions. 2. In rabbit 5 suprarenalectomy had been performed several months before the first dose of uranium was injected.

However, I have examined the aortas and measured the blood pressure of nineteen rabbits on which suprarenalectomy was performed by Dr. Rogoff,[31] and I have not found arteriosclerosis in them. This experiment is instructive, however, in that a severe arteriosclerosis (of the type caused by epinephrine hydrochloride) was developed under the influence of uranium after the main source of epinephrine (the suprarenals) had been removed. 3. The other complicating factors are the mixed intoxications, lead, radium and vanadium, purposely produced (with the exception of rabbit 17), as explained in the introductory remarks. From this point of view, the positive experiments appear as shown in table 5.

The only conclusion to be drawn is that commercial preparations of uranium nitrate are in themselves sufficient to induce arteriosclerosis in the rabbit. Other conclusions as to the predisposing or inhibiting effect of certain metals on arteriosclerosis caused by uranium are made

Table 5.—*Results of Positive Experiments*

Substance or Substances Used	Number of Rabbits	Arteriosclerosis Positive	Arteriosclerosis Severe
Uranium alone (all samples)	24	14	7
Uranium and lead	2	2	1
Uranium, lead and radium	4	0	0
Uranium and radium	12	2	1
Uranium and vanadium	2	1	1
	44	19	10

illusory by the great variability in the response of the animals and the small number of the experiments.

It seems to me that here there is a good field for a genetic study of arteriosclerosis. By proper selection and interbreeding along the lines of the already classic work of Maud Slye on the cancer of the rat, it might be possible to throw light on the inheritance of the susceptibility to arteriosclerosis. When one has control of this factor, the experimental study of arteriosclerosis will become less erratic than it is at present.

BLOOD PRESSURE

References to the method used in these experiments for the measurement of the blood pressure are given in the section on "material."

The systolic blood pressure was measured almost daily in thirty-two rabbits. Toward the end of the investigation, however, the measurements were made every four days, as the result had been so consistently negative. It is estimated that the number of blood pressure readings

31. Rogoff, J. M., and Dominguez, R.: J. Metab. Research **6**:141, 1924.

taken in these animals during the intoxication period is approximately 40,000. The results of these observations may be summarized as follows:

1. Changes in blood pressure did not occur under the influence of radium (rabbits 56 and 57), lead (rabbits 60, 61, 62 and 63) or vanadium (rabbit 65). A summary of the clinical history of rabbit 62, with a somewhat detailed account of the blood pressure, has been given previously.[32]

2. Changes in blood pressure did not occur in acute lead poisoning. Rabbit 59 was given 1.7 Gm. of lead acetate by stomach tube in seventeen days. It died two days after the last dose, in convulsions. The blood pressure oscillated between 120 and 140 mm. of mercury during this time.

3. Changes in blood pressure were not found in acute intoxication caused by uranium (rabbits 1 and 23). The control period in these animals was 186 days.

4. Under the influence of injections of uranium the blood pressure, in general, lowered.

Figure 9 illustrates what is meant by a lowering of the blood pressure. Suprarenalectomy had been performed on this animal (rabbit 5) a year before. The part of the graph from Aug. 12, 1924, before suprarenalectomy, to Dec. 4, 1924, nine weeks after the second operation, has been reproduced.[33] The blood pressure of rabbits 7, 9, 16, 18, 20, 46 and 50 behaved similarly to that of rabbit 5.

The blood pressure was low to start with and remained low throughout the experiment in rabbits 8, 25, 28, 33 and 36. In these five animals the blood pressure was rarely higher than 100 mm. of mercury. In rabbits 4, 6 and 37, the pressure lowered soon after the beginning of the intoxication and then slowly recovered its original level (below 120 mm. of mercury) toward the end of the experiment. Rabbit 3 maintained its initial level of pressure (between 130 and 140 mm. of mercury, occasionally higher) during the whole course of the experiment; the pressure sank rather critically below 100 mm. of mercury two days before death. In rabbit 11 the pressure fell when pneumonia developed. No change took place in rabbit 2, the pressure oscillating between 121 and 141 mm. of mercury.

Finally, in rabbits 21 and 22 the blood pressure increased from 90 to 130 mm. of mercury during the lead and radium intoxication, and then lowered as the experiment with uranium began. The twenty-

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32. Dominguez (reference 4, second part, "Blood pressure in pathological conditions," rabbit 487).

33. Rogoff and Dominguez (footnote 31, fig. 4, rabbit *D* 65).

one experiments are, then, uniformly consistent, and show that in chronic intoxication caused by uranium—varying within a wide range both in duration and in amount of uranium—the blood pressure either falls or remains about the preintoxication level.

5. There is one outstanding exception to this behavior of the blood pressure (rabbit 17). The graph is given in full (fig. 10). From *A* to *B* the animal was given lead carbonate by mouth. At *B* lead was discontinued, as explained in the introductory remarks and the section on general results. Three months later, on Sept. 3, 1924, the first injection of uranium was given (arrow in graph). From the fifth injection on, the blood pressure oscillated not only at higher levels than ever before, but in the vicinity of physiologic limits.[4] In the last

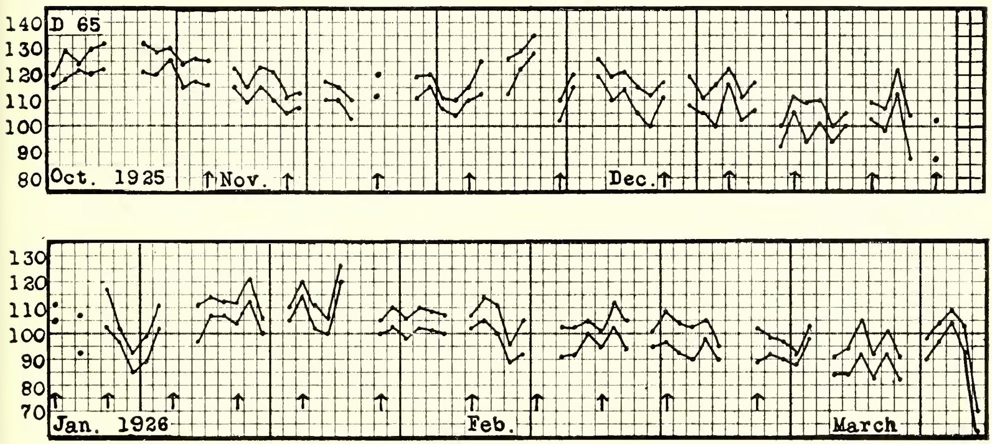

Fig. 9.—Blood pressure curve of rabbit 5. Arrows at bottom represent injections of uranium. The animal died on March 12, during the night. In this and figure 10, the ordinates represent blood pressure, millimeters of mercury; the abscissas, time, days; the upper curve, the highest readings of individual days, the lower curve, the lowest readings; the space between curves, daily oscillation of blood pressure. Daily readings numbered at least 10.

twenty days, when the animal was obviously losing ground, in spite of the cessation of injections of uranium, the pressure was still higher. It must be realized that this was the first experiment performed with uranium, when it was not known that an arteriosclerosis such as was found in this animal could be obtained, and, therefore, that this observation is as unbiased as it is possible to make one. As a matter of fact, all the work reported in the preceding pages was done to duplicate this blood pressure effect, without success.

As previously shown,[28] of ninety normal rabbits the blood pressure of which had been measured for varying periods (including the normal

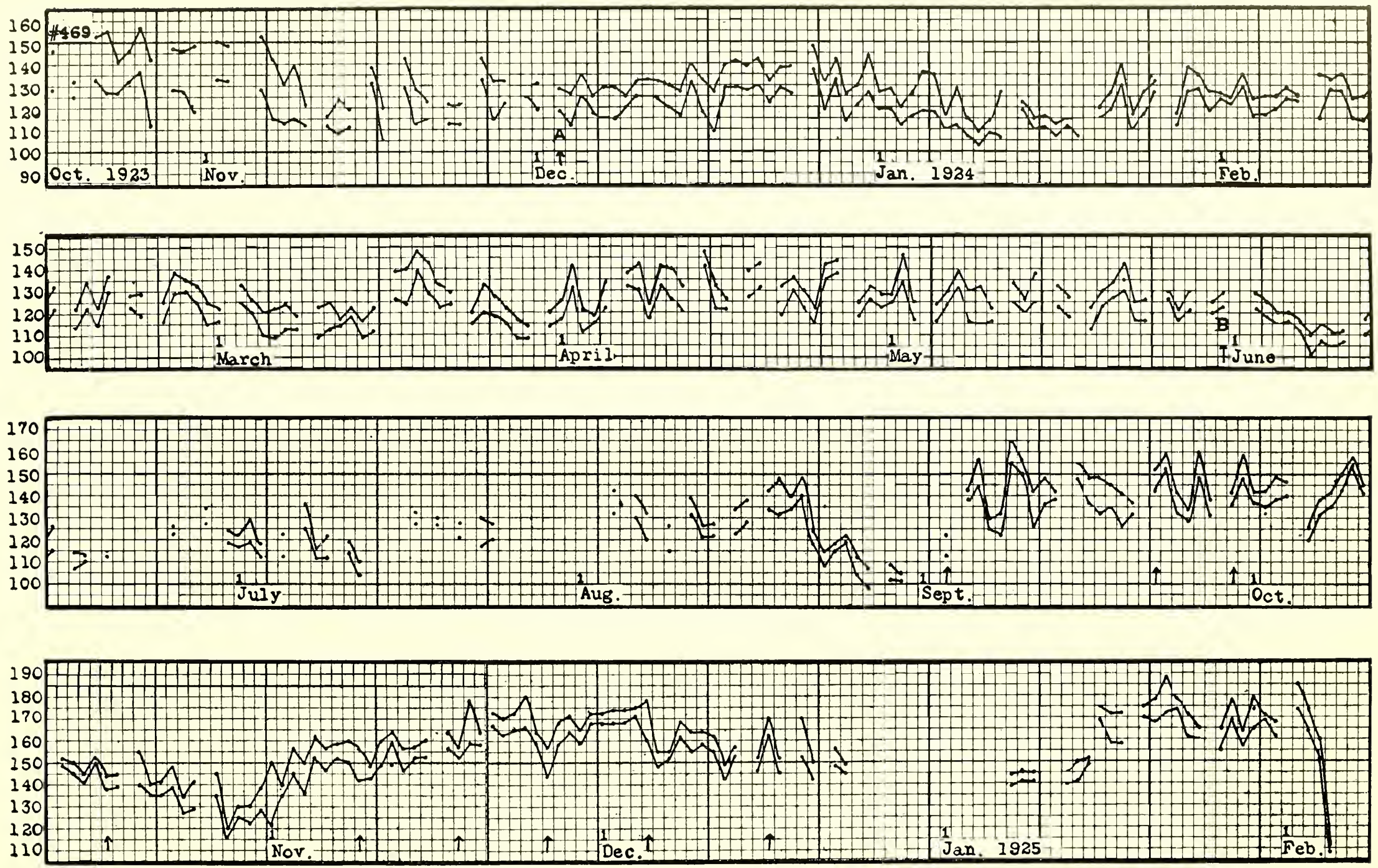

Fig. 10.—Blood pressure curve of rabbit 17; *A* indicates time when lead poisoning was begun; *B*, time when lead poisoning was discontinued; arrows at bottom, from September 3 on, represent injections of uranium. The animal was found dead on February 6.

period of the thirty-two animals of this report), only one (rabbit 489) compares with rabbit 17 under discussion.

The behavior of the pulse rate of this animal in the different periods of the experiment is shown in table 6. As shown in figure 10, period I extends from beginning to *A;* period II from *A* to *B,* that is, during the administration of lead; period III from *B* to the first arrow; period IV from the first arrow to death, that is, during the intoxication caused by uranium. Period V represents the intervals of highest pressures, from November 11 to December 11, and from January 15 to February 3, together. On February 4 and 5, the two days immediately before death, the pulse rate was 240 and 280, respectively.

This case, therefore, satisfies the criterion given previously [28] for a pathologic rise in blood pressure in the rabbit, namely, blood pressure in the neighborhood of 170 mm. of mercury, with a concomitant pulse rate below 200 per minute.

Table 6.—*Distribution of Pulse Rate of Rabbit 17 in the Different Periods of the Experiment*

Pulse Rate, Beats per Minute	Number of Counts in Period I	II	III	IV	V
280 to 299	..	..	..	1	..
260 to 279	3	..	..	..	..
240 to 259	8	..	..	1	..
220 to 239	20	1	3	..	..
200 to 219	**24**	11	15	17	2
180 to 199	15	58	**17**	30	9
160 to 179	10	**80**	9	**55**	**27**
140 to 159	..	21	8	18	7
120 to 139	..	9	2	5	3
	80	180	54	127	48

RELATIONS OF LESIONS TO OBSERVATIONS ON BLOOD PRESSURE

The fact that in intoxication caused by uranium the blood pressure either is unaltered or lowers has the following consequences, when brought into correlation with the anatomic lesions: 1. A severe arteriosclerosis may be produced without any elevation of the blood pressure (rabbits 3, 4, 5, 36 and 46, figs. 1, 2, 3 and 9). 2. A severe arteriosclerosis of the aorta and main arteries is not necessarily accompanied by a high blood pressure. 3. Nephritis of all degrees, from acute swelling down to the stage of granular contraction, caused by uranium, is not accompanied by high blood pressure (twenty-three animals). 4. Slight calcification of the interlobular arteries of the kidney and marked calcification of Bowman's capsules are not necessarily accompanied by a high blood pressure (rabbit 4, fig. 7). 5. The negative effect on the blood pressure is the same, whether the arterial calcification occurs in rings (rabbit 3), in plaques (rabbits 4, 36 and 46) or in both (rabbit 5).

The second consequence does not assume that uranium is responsible for the arteriosclerosis. It would, therefore, remain, even if future work were to deny the part played by uranium in this arterial calcification.

The first and second consequences do not support the mechanical theory of arteriosclerosis.

The exceptional case of rabbit 17 remains for consideration. The lesions in the aorta and main arteries differ from those in the other cases (rabbits 3 and 49, for example) only in degree and extent. The lesions in the kidney differ from those in rabbit 4 in two respects: in the severity of the calcification of the small vessels (figs. 5, 6 and 7 should be compared) and in the glomerular destruction. Many of the glomeruli were completely obliterated, particularly in the outer parts of the cortex, and were the seat of an abundant deposit of calcium. The vessels of the other organs show slight or no change. In certain parts of the heart, the coronary arteries show a discrete calcification involving only a small fraction of the circumference (hematoxylin-eosin preparations). In the thyroid gland, similar discrete changes were seen in sections near the hilum. The spleen and liver were normal. In the brain, many sections from all parts of the organ, especially from the medulla, did not show vascular disease. The organs of this animal had been kept in alcohol to preserve the deposits of calcium, and the brain was examined recently, in view of the report of Bordley and Baker.[34] The facts in this case are, therefore, consistent with the theory that hypertension is intimately connected with injury to the vascular apparatus of the kidney.

LITERATURE ON ARTERIOSCLEROSIS CAUSED BY URANIUM

The only mention I have found in regard to a relation of uranium to arteriosclerosis is the following, from Saltykow's [35] review: "Erwähnt sei hier noch, dass Fischer bei intravenöser Injektion verschiedener Substanzen positive Resultate erhielt, *wenn auch nicht so regelmässig und hochgradig wie bei Adrenalin;* es sind dies: Salzsäure, Phosphorsäure, Milchsäure, phosphorsaurer Kalk, Kaliumbichromat, *Urannitrit,* Chloralamid, Sublimat, Phloridzin, Trypsin, Pepsin, Thyreoidin, Mamma siccata, Kochsalzlösung." (It may be noted, further, that Fischer, on intravenous injection of various substances, obtained positive results, *although not so regularly and not of such a marked character as with epinephrine.* The substances employed were: hydrochloric acid, phosphoric acid, lactic acid, calcium phosphate, potassium bichromate, *uranyl nitrite,* chloralamide, mercuric chloride, phlorizine, trypsin,

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34. Bordley, J., and Baker, B. M.: Bull. Johns Hopkins Hosp. **38**:320, 1926.
35. Saltykow (footnote 18, p. 373).

pepsin, iodothyrine, mamma siccata, sodium chloride solution.) (Italics mine.) I regret that I have not read the original work of Fischer. Judging from the phrase in italics, it seems to me that he did not see in his experiments the profound changes I have described here, since these lesions are at least as severe as those produced by epinephrine. My lesions show, besides, some morphologic differences from sclerosis (rings) caused by epinephrine, and are accompanied, furthermore, by frequent involvement of the main arteries of the body.

Dickson [29] did not find any arterial lesions in his experiments on chronic nephritis caused by uranium. He [36] says, "A very striking feature of chronic uranium nephritis is that, even in the advanced cases in which the kidneys are hard and granular, and in which the greater part of the parenchyma is replaced by dense fibrous tissue, there is no demonstrable lesion in the vessels of the kidneys or of the peripheral vascular system." A critical examination of his reports of cases, in the light of table 1, shows the following facts:

Twelve rabbits received an occasional sublethal dose of uranium nitrate. The experiments lasted from thirty-nine to 543 days, the number of injections varied from two to six and the amount of uranium varied from 6 to 25 mg. These experiments do not compare with mine, and some may be discarded, since I have discarded eight of mine in which fewer than eight injections were performed. Four rabbits received from thirty-two to thirty-four injections, and as many milligrams of uranium, in an interval varying from 193 to 457 days. In these four the results may be compared with mine. In only two is the aorta mentioned (rabbits 11 and 14). It was "apparently normal."

Discrepancy is not found, then, between Dickson's negative observations and my positive results.

SUMMARY AND CONCLUSIONS

This paper contains observations on pathologic processes and blood pressure in fifty-two rabbits subjected to intoxication caused by uranium. Some of these animals received lead, radium or vanadium in combination with the uranium or before it was administered. Fourteen additional rabbits served as controls for the administration of the lead, uranium and vanadium. The general results of the investigation are presented in table 1.

Positive results were obtained only in animals which received uranium. The lesions consisted of: (1) arteriosclerosis of the medial calcification type, involving the aorta, pulmonary artery and aortic branches; (2) renal tubular necrosis, with and without calcification of the small vessels and of the Bowman's capsules; (3) necrosis and calcifi-

36. Dickson (footnote 29, p. 584).

cation of the muscular coat of the stomach, and (4) necrosis and calcification of the striated muscles. These lesions are described in detail and then discussed from the standpoint of general response, severity, frequency and mutual relationships.

A section is devoted to the question of "spontaneous" sclerosis and sclerosis caused by epinephrine hydrochloride.

The blood pressure was studied in thirty-two rabbits by means of the van Leersum method (about 40,000 blood pressure readings). Five photographs of anatomic lesions, three photomicrographs of the renal lesions and two blood pressure curves (three graphs) are given.

The following conclusions are reached:

1. Changes in blood pressure do not occur in intoxication caused by radium, lead and vanadium.

2. Acute lead poisoning is not accompanied by a rise in blood pressure.

3. Changes in blood pressure are not observed in acute intoxication caused by uranium.

4. The blood pressure in chronic intoxication with uranium becomes lower or, in general, remains unaltered.

5. Nephritis of all degrees, from acute swelling (conclusion 3) to granular contraction, caused by uranium, is not accompanied by high blood pressure.

6. A severe arteriosclerosis (medial calcification type) of the aorta and main arteries may develop without any elevation of the blood pressure.

7. Conversely, severe arteriosclerosis of the aorta and main arteries is not necessarily accompanied by a high blood pressure. Similar conclusions have been reached in experimental atherosclerosis (intimal fatty deposit).[37]

8. These experiments do not support the mechanical theory of arteriosclerosis.

9. Calcification of Bowman's capsules, with slight calcification of the small vessels of the kidney, is not necessarily followed by a rise in blood pressure.

10. One animal, in which the calcification of the small vessels of the kidney and the glomerular destruction were pronounced, developed a pathologically high blood pressure.

11. The results obtained agree with the idea of an intimate relationship between the vascular apparatus of the kidney and high blood pressure.

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37. Dominguez, R.: J. Exper. Med. **46**:463, 1927.

I wish to acknowledge my indebtedness to Dr. G. N. Stewart for encouragement and helpful criticism during the course of the work; to Dr. J. M. Rogoff for valuable assistance and advice and to Miss W. Kuenzel, of the department of anatomy, for her skillful photographic work. The radium standard used was lent by Dr. E. Karrer, to whom I am also indebted for suggestions concerning electroscopic determinations. The electroscope was made in the laboratory after the pattern of one kindly loaned to me by Mr. C. D. Hodgman, of the Case School of Applied Science. To Dr. H. Goldblatt, I owe services too many and too varied to be enumerated. He and Dr. A. Moritz looked after the experiments during my absence in the summer of 1925.

Reprinted from the Archives of Pathology
April, 1928, Vol. 5, pp. 577-606

Reprinted from THE AMERICAN JOURNAL OF PHYSIOLOGY
Vol. 90, No. 2, October, 1929

KARRER, ENOCH and J. M. ROGOFF (CLEVELAND). **A Method of Blood Transfusion by Means of Rubber Tubes Vulcanized on the Blood Vessels.**

Experiments are reported in which blood transfusion was carried out on living animals by means of rubber tubing vulcanized on the vessels. The technique is described and the advantages of the method are discussed.

Reprinted from THE AMERICAN JOURNAL OF PHYSIOLOGY
Vol. 90, No. 2, October, 1929

STEVENS, H. C. and J. M. ROGOFF (CLEVELAND). **Some Effects of Denervation on Muscular Contraction.**

Simultaneous records were made of the contraction of the two gastrocnemii of the frog, in one of which the sciatic nerve was previously severed (at intervals of 7 to 21 days). Certain constant differences in response of the innervated and denervated muscles were observed. The denervated muscle showed a slight degree of contracture; quicker relaxation; a slower response to the stimulus; and slight tendency to staircase. The bearing of these observations upon the mechanics of muscular contraction is discussed.

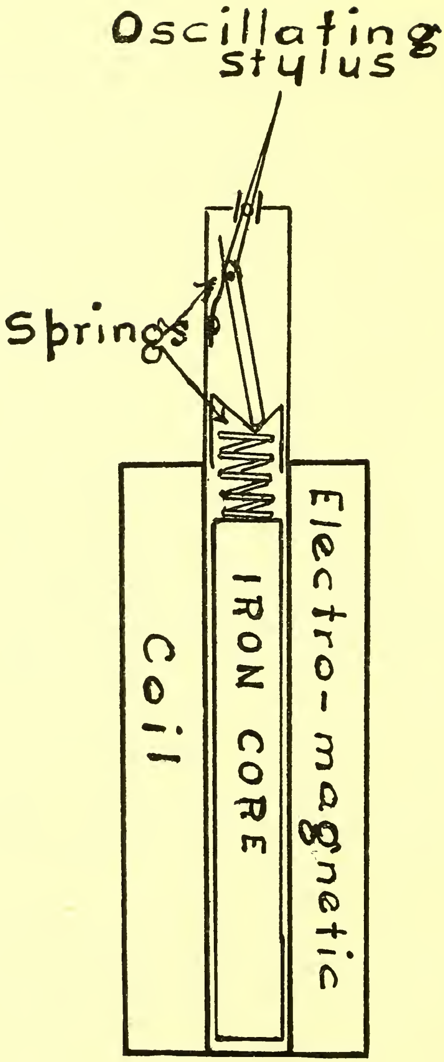
Reprinted from SCIENCE, July 27, 1928, Vol. LXVIII, No. 1752, pages 87-88.

A SIMPLE AND ACCURATE TIME MARKER

THIS time marker takes advantage of the fairly constant frequency of the ordinary alternating current lighting circuit. There are three forms of it that we have considered. The first and simplest consists of a single light reed whose period of vibration is the same as the frequency in the A.C. line. The reed is actuated by a suitable small electromagnet.

The second form of this time marker, which is depicted in Fig. 1, may be looked upon as a modification of the synchronous reed. It consists of a system of levers and a movable soft iron core in an electromagnet. One or more springs, helical or linear, furnish restoring forces. This second form may be designed to be quite powerful. The mechanism which we have modified and used was obtained in the market in the shape of an electric safety razor. It is shown schematically in Fig. 1. These time markers, which may be constructed in the form of a pencil, will require little space and may be readily mounted with any desired freedom of adjustment. It is not easy to construct such a system as that shown in the drawing to have a natural frequency equal to that of the A.C. line. Furthermore, its wave form may not very closely approach a simple sine wave. There are advantages and disadvantages in having harmonics present in the time graph.

Applying a somewhat different mechanism from the two forms just described for the registration of time intervals of the alternating circuit, we have attempted to employ as the third form of time marker a synchronous motor such as is used in the electric timepiece which is sold under the trade name of Telechron. This apparatus can be made not only to


Oscillating stylus
Springs
Electro-magnetic
IRON CORE
Coil

indicate the smallest time interval that may be estimated on the drum, but also, by proper gearing, it can be made to indicate on the graphic record each tenth of a second or other desired interval. It is this form which we hope to set forth in greater detail in the near future.

We would like to emphasize that such synchronous A.C. timing devices are extremely convenient and sufficiently reliable for many ordinary laboratory purposes. In the Cleveland district the potential cycle known as the 60 cycle circuit is constant within a quarter of a cycle from day to day. This means that the time intervals might be accurate to within 1/240 of a second from day to day. For a matter of a few hours during the course of an experiment the time as indicated by such a synchronous device is considerably more accurate. Depending upon the speed of the drum, upon which the time graph is made, the time may be read to something certainly less than 1/280 of a second, possibly quite readily to within 1/600 of a second. This accuracy of timing refers to relative time intervals and not to any accuracy of synchronism with ordinary clocks. The so-called "60 cycle circuit" may actually have been controlled at any frequency other than 60 per second, as was the case in our local circuit, where the frequency was 59.6.

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Vol. XIV, No. 3, P. 265, December, 1928.

A HEAD CLAMP FOR DECEREBRATE ANIMALS*

BY ENOCH KARRER, PH.D., AND H. C. STEVENS, PH.D., M.D., CLEVELAND

IN THE course of some recent work on the measurement of muscular tension in decerebrate cats, it was found convenient to mount the preparation on a canvas frame in which there were openings through which the legs were thrust. To hold the head upright, in order to facilitate respiration, it was found advantageous to construct the head clamp which is here pictured in Fig. 1 and described. Two bars are hinged together, the one, A, being straight and having riveted on its end a small piece of wood, B, or rubber from the tread of an automobile tire; the other bar, C, is curved along its length in

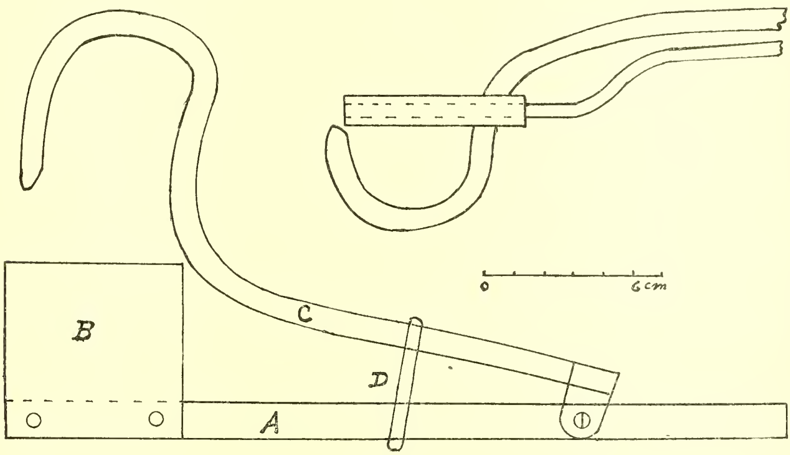

Fig. 1.

several directions and is hooked on its end to fit the nape of the neck under the occipital protuberance. When ready to use the head clamp, the bars are opened like a pair of shears and the wood (or rubber) piece is inserted between the teeth and the hook is placed about the neck.

The two bars are held together by the band, D, which by being forced along the bars, locks them together. The end of the head clamp is fastened to a vertical rod by means of an ordinary laboratory clamp. The grip of the head clamp is so secure that a decerebrate animal could be freely suspended by the head, if necessary, without mechanical interference with respiration or circulation. A head holder of this type could be usefully applied to the human cadaver for the purpose of holding the head rigid while sawing through the skull, in the performance of a postmortem examination.

*From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, Cleveland, Ohio.

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A SPECIAL KNIFE FOR DECEREBRATION*

BY ENOCH KARRER, PH.D., AND H. C. STEVENS, PH.D., M.D., CLEVELAND

THE instrument here described is designed for the decerebration of cats and animals of like size through a trephine opening in the skull. It is especially designed to produce the particular type of decerebration known as the thalamus preparation. The instrument is drawn in Fig. 1, in which 1 is a

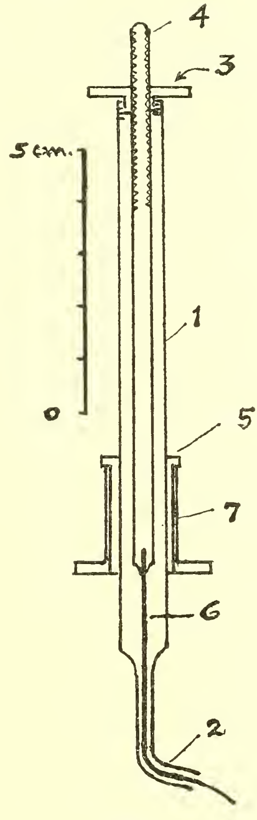

Fig. 1.

small steel tube having at its one end a flat and smaller portion 2, with relatively sharp edges, and having on its other end a rotatable but not translatory nut, 3. Through the nut 3, passes a portion of a screw 4, extending down into the tube 1, and terminating in a flat portion of steel, or other material, 6 whose edges may be sharpened to enable it to cut through the tissues inside the cranium. This flattened portion is also slightly curved downward. The whole tube 1 may be rotated in a sleeve 7 where tube 5 affords a bearing that

*From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, Cleveland, Ohio.

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may be slid along tube 1. The extensible knife-edge 6 is withdrawn by manipulation of screw 4, and the end 2 is inserted into the cranial cavity through an opening in the skull situated in the median plane approximately 1 cm. caudad to a straight line joining the outer angles of the posterior borders of the two orbits of the eyes. The depth of introduction is determined by the position of the sleeves 5 and 7. In case of average size cats this distance should be from 1 to 1.6 mm. The extensible knife-edge 6 is then caused to project by turning the nut 3 until the end of it is felt to be against the cranial wall. Then the whole tube is rotated in 7 to cut the tissues that will fall on the surface described by the cutting edge. The knife when manipulated in accordance with the directions given above passes through the brain just cephalad to the optic thalamus. The projecting cutting edge, 7, is best made of rustless steel or other material as sheet molybdenum. When made of high carbon steel, rusting is serious through the narow tube 2.

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Vol. XIV, No. 2, P. 171, November, 1928.

A TENDON CLAMP FOR MEASUREMENTS OF MUSCULAR TENSION*

BY ENOCH KARRER, PH.D., AND H. C. STEVENS, PH.D., M.D., CLEVELAND

IN MEASUREMENTS of muscular tension, the muscle is attached to the dynamometer or weight by means of its tendon. The connection is usually made by inserting a hook through the tendon. This method is objectionable for the reason that a hook tends to slip, unless special precautions are taken, and, as tensions become great, the hook cuts into the tissues. In certain prob-

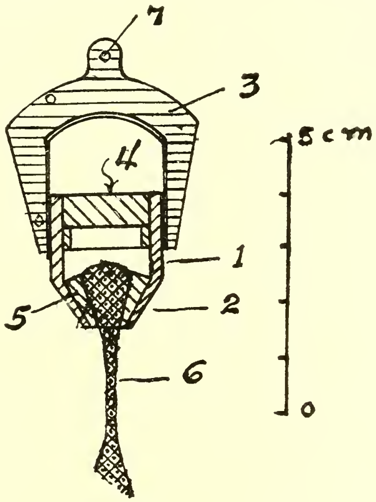

Fig. 1.

lems in which exact measurements of muscular tension are required, such as the problem of the pluri-segmental innervation of muscles, the maximal force of the muscle is exerted with a very slight shortening of the muscle. One-tenth of a millimeter may represent many grams. It is important, therefore, to be able to connect the tendon to the dynamometer in such a manner that there will be no yielding due to slipping or cutting when the muscles exert their maximal force. To meet the need for a mechanical mode of attachment which will neither slip nor cut the tissue, a tendon clamp has been devised.

This consists of a small brass cylinder, 1, which is straight for a portion of its length and conical on one end, 2. It has attached to it a yoke, 3, built of thin sheet metal in the fashion of a structural T-beam to give strength with lightness. If thought desirable small holes may be drilled in the yoke

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for suspending the tendon holder in the horizontal position in case the passive extension of the muscle is not sufficient to so maintain it. This yoke has a hole, 7, for attaching to the dynamometer or counter weight system, by clevis or other means. In the straight end of the small brass cylinder just mentioned, is a nut, 4, which may be unscrewed and removed entirely while the tendon is being inserted. There are two conical aluminum jaws, 5, the two halves of a cone, which fit into the conical end of the brass cylinder, 2. The tendon, 6, which is desired to be clamped is inserted through the hole in the conical end of the brass tube and the aluminum jaws, 5, are inserted from the rear which is open after the removal of nut, 4. This latter nut is then inserted and screwed down tightly against the distal surface of the bone on which the tendon terminates. We have found that rather than screwing nut, 4, onto the tendon, the insertion of a stiff rubber block which bears against the tendon and against the brass nut, 4, is more satisfactory in that it cannot mutilate the attachment of the tendon. It is obvious now that the tendon is securely clamped over a considerable portion of its surface between the aluminum jaws which have a tendency to tighten the greater the force which acts upon the tendon.

Reprinted from THE AMERICAN JOURNAL OF PHYSIOLOGY
Vol. 85, No. 2, June, 1928

The measurement of muscular tension and its bearing on plurisegmental innervation. E. KARRER and H. C. STEVENS.

The distribution of the constituent, effector, fibers of a nerve to the muscle fibers of a striated muscle may be conceived of in several ways: *a*, one nerve fiber to one muscle fiber; *b*, branches of the same axone to several muscle fibers; *c*, double innervation in which two different axones innervate the same muscle fiber. Experiments made to determine which of these possibilities is the true one may be classified as anatomical and physiological.

Physiological observations depend upon the measurement of *a* muscular tension; *b* action current of muscle; *c* heat developed during muscle contraction. The physiological method requires a muscle-nerve preparation in which the same muscle is innervated by two nerve sources, such as the L_8 and L_9 of the frog which unite to form the sciatic nerve, and the gastrocnemius muscle. The muscle response is measured when L_8 is excited alone: then L_9 alone and finally by both L_8 and L_9 together. The problem may be stated by using letters to stand for the nerves and the muscular response. Let a stand for one nerve; let b stand for the other nerve; let T_a stand for the tension developed when a is excited alone; let T_b stand for the tension developed when b is excited alone; let $T_{(a+b)}$ stand for the tension developed when a and b are excited together. The problem to be solved by experiment is whether $T_a + T_b \gtreqless T_{(a+b)}$. The results of physiological investigations show that measurements of heat and action current are additive. Resorting to symbols and letting H stand for heat (or action current)

$$H_a + H_b = H_{(a+b)}$$

Most of the reported measurements of tension are not additive. $T_{(a+b)}$ is from 15 per cent to 50 per cent less than $T_a + T_b$. Hence some investigators, relying on these observations have concluded that a certain percentage of the muscle fibers are doubly innervated by axones from both a and b. Their conclusion is based upon the assumption that a muscle fiber would exert the same force if it were excited by two nerve impulses as it would if excited by one. Analysis of the mechanical conditions under which muscular tension is measured has led us to believe that certain errors in technique tend to make $T_{(a+b)}$ small as compared with $T_a + T_b$ By eliminating these errors we find that Tension is ninety-six per cent additive. The lateral head of the gastrocnemius of decerebrate cats was used in these experiments. An apparatus so constructed as to minimize errors in the measurement of tension is described.

Reprinted from The American Journal of Physiology
Vol. 88, No. 4, May, 1929

THE MEASUREMENT OF MUSCULAR TENSION AND ITS BEARING ON PLURI-AXONAL INNERVATION

H. C. STEVENS and ENOCH KARRER

From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University

Received for publication February 9, 1929

The problem of the ultimate mode of distribution of the constitue
fibers of a motor nerve to the constituent fibers of a skeletal muscle h
interested anatomists and physiologists for many years. The attack
the problem by modern experimental methods may be said to have beg
with the publication by Johannes Gad (1882) of his classical paper on t
relations between nerve, muscle and center. Since then the subject h
been studied both by anatomical and physiological methods. The liter
ture on the question has been recently reviewed by Fulton (1926) and
Weed (1927). The general conclusion, reached by anatomists, appears
be that in some instances branches of the same axone may innervate sing
several muscle fibers and in other instances, doubly innervate certa
muscle fibers. The problem has usually been stated with reference to t
unisegmental or plurisegmental origin of the nerves. However, the que
tion as to whether muscle fibers are doubly innervated may be discuss
without reference to the spinal origin of the nerves. We have chosen
consider the problem from this point of view using the terms *uni-axon*
and *pluri-axonal* innervation to designate respectively the anatomic
conditions in which a muscle fiber is innervated by one nerve fiber and
two or more fibers derived from different axones. Possible relationshi
between axonal and segmental innervations are illustrated in figure 1.

We were induced to take up the study of this problem because of certa
questions which arose in connection with the conduction of the ner
impulse through a zone of narcosis. It became evident, when repeati
some of Adrian's experiments on the conduction of nerves narcotized
alcohol, that the determination of the end point of the reaction by mea
of the muscular contraction could not serve as a true indication of t
narcosis of the nerve trunk, fiber by fiber, unless a one to one relationsh
exists between nerve fiber and muscle fiber. For it is obvious, to assu
the extreme case, that if all the fibers of a muscle are doubly innervate
50 per cent of the nerve fibers composing a nerve trunk could be narcotiz
without affecting the response of the muscle, provided that both of t

innervations were equally potent to excite the muscle fiber. Since, in experiments of this sort the muscle response is employed to indicate what is going on in the nerve, it would seem to be necessary first to understand the nature of the innervation of the muscle.

The special anatomical condition required by the physiological method is a muscle which derives its nerve supply from two separate sources such as the gastrocnemius of the frog, which is innervated chiefly from the 8th and 9th lumbar roots or the crico-thyroid muscle which is innervated by the middle and superior laryngeal nerves. It may be pointed out that it is possible to split a motor nerve close to its point of entrance into the muscle and so create the anatomical condition required by this method.

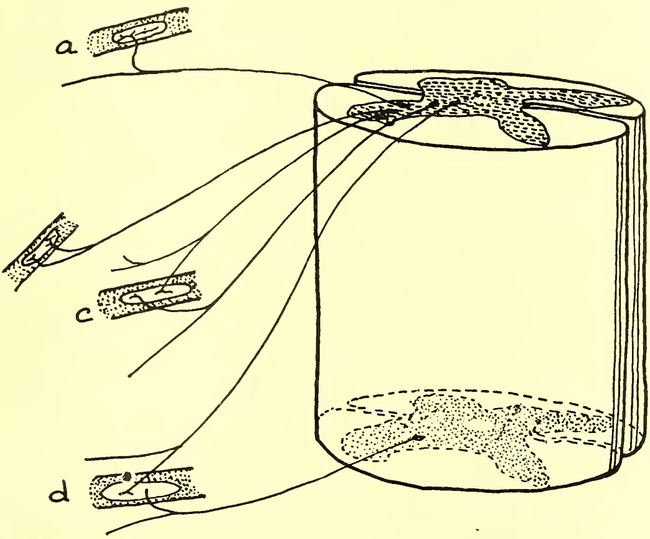

Fig. 1. *a*, uni-axonal, unisegmental; *b*, double innervation from one axone; *c*, pluri-axonal, unisegmental; *d*, pluri-axonal, plurisegmental.

Since the two sets of axones enter the muscle grossly at the same point, it may be suggested that the possibility of overlap might be greater than if the nerves enter at different points as do the branches of the sciatic which supply the gastrocnemius. This procedure makes possible the use of any motor nerve and any muscle.

With such an anatomical mechanism, the response of the muscle measured as mechanical tension, action current or heat is determined when the muscle is excited by stimulation of one nerve alone, then of the other alone and finally of both together. Three measurements are thus made and their bearing upon the pluri-axonal question depends upon the relation of these three quantities to one another. The following symbols represent the quantities and their relations. Let *a* stand for one, and *b* the other

nerve source; R for the response of the muscle (mechanical tension, action current or heat); Ra, Rb and Rab the response of the muscle when excited respectively by stimulation of a and b separately and simultaneously. The sum of Ra and Rb then is Ra + Rb. There are three possible quantitiative relations between Ra + Rb and Rab viz., Ra + Rb $\gtreqless$ Rab.

The results reported by investigators of these three alternatives have varied with the experimenter and the type of measurement made. In general the measurement of action current and heat has yielded approximate equality of Ra + Rb and Rab. Measurement of tension, on the other hand, has in general resulted Ra + Rb > Rab. There have been some notable exceptions, as in the early experiments of Gad (1882) which were made with a Fick isometric lever, and in those of Quednau (1926). Usually, however, Rab has been reported from 11 per cent to 50 per cent less than

TABLE 1

Tension in kilograms

	ORDER OF STIMULATION	L	R	L + R	B	$\frac{\text{B}}{\text{L and R}}$
						per cent
Experiment 1	L-R-B	2.070	3.560	5.360	5.090	90.4
Experiment 2	L-R-B	1.141	3.080	4.221	4.240	100.4
Experiment 3	B-L-R	0.933	2.590	3.523	3.715	105.4
Average						98.7

Ra + Rb. Such results have been interpreted to mean that a certain percentage of the muscle fibers are doubly innervated by fibers from both a and b. This inference implies the assumption that a muscle fiber doubly innervated behaves like one singly innervated, irrespective of whether it be stimulated in any one of several ways, such as through either one of the nerve fibers alone or through both simultaneously, or through both alternately.

A brief résumé of the literature will suffice to show that the results of measurement of muscular tension have, in general, not been additive while measurement of action potential and heat yield approximate summation.

Gad (1882) using a Fick isometric lever and the gastrocnemius of the frog found that tension was 100 per cent additive. Exner (1885) employing the crico-thyroid muscle which he found to be doubly innervated by the superior and middle laryngeal nerves, attempted to determine the amount of overlapping by section of one nerve and observing the subsequent degeneration in the muscle. His results were incon-

clusive. Lederer and Lemberger (1907) using the same preparation and the Fick lever obtained 100 per cent additivity of the muscular tension. Their results with the flexor digitorum of the rabbit failed to give summation. Agduhr (1916, 1919) renewed interest in the problem by histological studies using the method of nerve degeneration and also by the measurement of tension. The latter experiments gave from 55 per cent to 60 per cent summation. Cattell and Stiles (1924, a, b) measured muscular tension in the gastrocnemius of the frog and obtained an additivity of about 70 per cent. Samojloff (1924, 1925) measuring simultaneously the action potential and the tension of the frog's gastrocnemius found 102.4 per cent for the former and 80 per cent for the latter. Katz (1925) obtained 77 per cent for tension and 101 per cent for heat in experiments made with the gastrocnemius of the frog. De Boer (1926) reported 100 per cent additivity in measurements of action potential of the gastrocnemius of the frog. Boyd (1926) attempted to correlate a low additivity (23 per cent with the triceps surae of the frog) with the degree of obliquity of the muscle fibers in pinnate muscles. Fulton (1926) making use of the torsion wire myograph and the gastrocnemius of the frog obtained a summation of 89 per cent. Quednau (1926) obtained 100 per cent additivity with the flexor digitorum of the rabbit. Cattell (1928) employing the sartorius of the frog measured tension, under isometric conditions, by means of the torsion wire myograph with the result that the combined tension varied from 53 to 88 per cent of the sum of the separate tensions.

In our experiments the lateral head of the gastrocnemius muscle of decerebrate cats was employed. Decerebration was performed by transecting the brain stem by means of a stiff wire (under ether anesthesia and after ligation of the carotid arteries), through a small trephine opening, except in some experiments where a thalamus preparation was desired, a special knife (Karrer and Stevens, 1928a) designed for this purpose was used. It is possible, in the cat, to separate the medial head of the gastrocnemius from the lateral and by ligating and amputating the former, to leave the lateral head intact with two branches of the tibial nerve entering it at different points. A thin band of muscle tissue along the median line of the dorsal surface of the muscle is severed. When there is bleeding at this point, it is controlled by ligation. The two branches *a* and *b* of the tibial nerve enter the muscle at different points, *a* entering on the medial surface of the muscle about 2 cm. from the proximal end, and *b* in the mid-line of the dorsal aspect about one centimeter from the origin of the muscle. The former innervates mainly the dorsal and medial portion of the muscle, the latter chiefly the lateral and inferior portion. This preparation presents certain advantages for the study of muscle nerve problems in mammals. Circulation in the muscle is little disturbed and the animal survives on the average for about six to eight hours. The sciatic nerve is accessible for several centimeters and the two branches from the tibial nerve which enter the lateral head can easily be freed, for a distance of 2 cm. from the muscle. The large femur by which the leg may be clamped and the large tendo achillis are useful for purposes of fixation and traction. To avoid loss of body heat and drying, electric lamps are placed under the animal and the muscle and nerve kept moistened with

Ringer's solution. With the electrodes placed in the middle of a portion of nerve about 2 cm. in length, current spread was tested by section of the peripheral end of one nerve and maximal stimulation of the central end of the same nerve to observe whether there was response through the intact nerve and its portion of muscle. It is essential to avoid such sources of error as insecure fixation of the femur, the slipping, lengthening, cutting and tearing at the hook which is inserted through the tendon. This hook is wrapped and bound to the tendon and bone by means of twine. The femur is exposed and firmly gripped by the teeth of the clamp which would penetrate any layer or cushion of tissues. The tendon and the opposing spring are attached to the pulley system by means of several strands of fine, flexible steel wire. The lever system magnified 5.47 times. Since, in our experiments, it was possible to record and observe, with reasonable

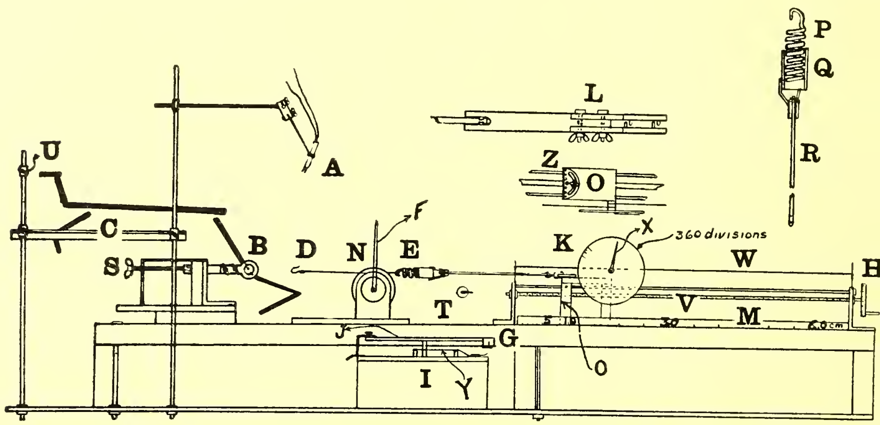

Fig. 2

certainty, approximately 0.1 mm., the muscle was isometric to within about 0.02 mm. at the moment when it exerted its maximal force.

Apparatus. The apparatus, which was developed after much preliminary experimentation necessary to overcome the many technical difficulties associated with measurement of muscular tension, is shown in some detail since with improvement of the apparatus we were enabled to obtain more satisfactory experimental results. The general arrangement of the various parts seen from the side is shown in figure 2, in which a decerebrate cat is represented by the heavy black line as lying belly down on a canvas frame *C* with its head supported by a head holder, *U* (Karrer and Stevens, 1928b). The femur is exposed and clamped in a bone clamp, *B* (insert *L*). Two such clamps are mounted side by side, separated sufficiently to permit the simultaneous use of both legs. Four

electrodes, of which one only, A, is shown, are available. The tendon is attached to the lever system, N, by means of a hook or tendon clamp (Karrer and Stevens, 1928c). The stylus, F, is adjusted to write on a drum (not shown) which is carried on the disc, J, of a phonograph motor, I. The nerves are excited by an automatic stimulator, G, consisting of spring brushes which make contact across two wires, Y, at each revolution. The duration of stimulation can be varied by lengthening these wires or changing the speed of the motor. A time marker, T (Karrer and Stevens, 1928d) giving a time interval of approximately $\frac{1}{120}$ second, is adjusted to write on the drum under the stylus and in alignment with the signal marker which is not shown. The contracting muscle is opposed by the adjustable spring, E, (insert PQR) one end of which is attached to the lever system, N, and the other to the traveling carriage, O (insert ZO, top view). Tension of the spring is increased or diminished by turning the screw, V, by means of the disc, H. Readings are made on the scale, M, for coarse, and on the dial, K, for fine adjustments. The pointer, X, of the dial is rotated by means of the wire, W, which encircles a cylinder mounted upon the carriage, O, to which the pointer is attached. Each degree on the dial corresponds to 0.12 mm. of travel of the carriage, O.

Early in these experiments it was found, as others have found, that the more nearly isometric the conditions were and also in general the stiffer the spring which was used to oppose the muscle, the more perfect was the additivity. This became quite striking while obtaining myograms with different springs and under different conditions. In figure 4 are plotted the elastic constants of the springs used (abscissae) and the per cent of additivity (ordinate) which was obtained. This graph shows that there is a high degree of correlation between stiffness of spring and high additivity of muscular response. At the moment when the maximal tension is recorded, the muscle is isometric to the same extent in both cases. The spring was adapted to the particular animal used in an experiment, by means of the following method. First a long spring (22 cm. long, stretching 1.55 cm. for each kilo) was used and, with an initial load of 50 grams to 80 grams, the maximal shortening of the muscle was determined with the least maximal contraction stimulus (L.M.C.S.). With the same spring, the maximal load of the muscle under isometric conditions was determined. Two limiting conditions were thus measured, viz., maximal shortening with minimal load and maximal load with minimal shortening. The third step was to calculate that length of the adjustable spring (fig. 2, E and PQR) which would be stretched a distance equal to the maximal shortening under minimal load by the maximal load determined under isometric conditions. For example, if the maximal shortening under the initial load was 1 cm. and the maximal force developed under isometric conditions was 10 kgm., we determined by calculation, from the

elastic constants, the length of the adjustable spring which would be extended 1 cm. under a load of 10 kgm. Springs selected in this manner gave good additivity and most of our observations were made by such springs. In five experiments, the spring was deliberately lengthened after good additivity had been obtained with a shorter spring. This change always resulted in poor additivity (cf. fig. 3 the points indicated by *S*). The results obtained in such experiments are not included in table 2.

To determine the range of experimental error inherent in this type of experiment, with our apparatus and technique, the entire lateral head of the gastrocnemius and the tibial nerve of each leg were used instead of one muscle and the branches of the nerve. With two separate legs, there can be no question of a common field of innervation. The sum of the tensions developed in the two muscles excited separately should approximately

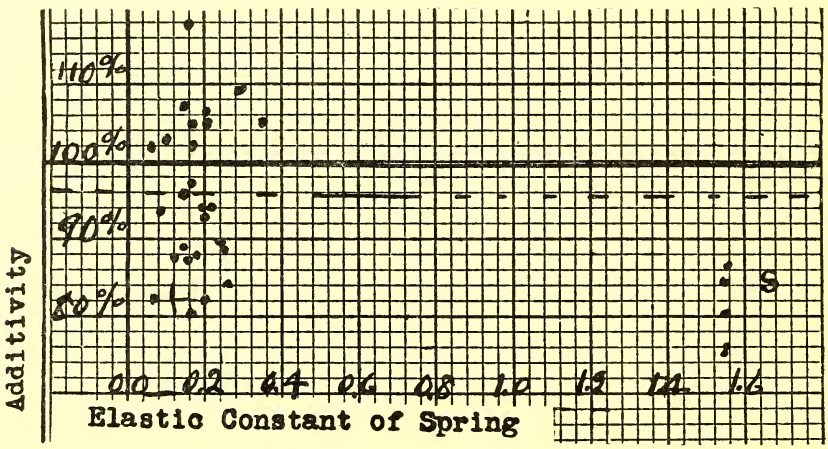

Fig. 3

equal the tension of the two muscles excited together. Ra + Rb should equal Rab. Typical results are given in table 1 where three measurements are recorded with the lateral heads of the right and left gastrocnemii of a cat. The letters L, R and B refer to the left, right and both lateral heads of the gastrocnemii, respectively. It will be seen that the range of experimental error is about 5.5 per cent.

In our experiments we first determined the least maximal contraction stimulus (L.M.C.S.). The stimulus which had a duration of 0.12 second was produced by the secondary coil of a Harvard inductorium, the primary of which was activated by a two volt storage battery. The L.M.C.S. was first obtained, the secondary coil usually requiring to be 11 cm. from the primary and tilted about 30° from the vertical. We next determined the optimal, initial, passive extension. This was accomplished by turning

TABLE 2

Tension in kilograms

DATE	ORDER OF STIMULATION	Ra	Rb	Ra + Rb	Rab	ADDITIVITY Rab/(Ra + Rb)
						per cent
December 5, 1926	ab-b-a	1.787	2.403	4.190	4.072	97.1
December 8, 1926	ab-b-a	0.470	0.170	0.640	0.700	109.3
December 10, 1926	b-a-ab	1.460	2.440	3.900	4.100	106.0
December 12, 1926	b-a-ab	4.330	6.380	10.710	9.260	86.4
	b-a-ab	4.420	6.020	10.440	9.360	89.6
December 19, 1926	b-a-ab	3.635	4.934	8.569	9.112	106.3
	b-a-ab	3.906	5.663	9.569	9.112	95.2
	ab-b-a	3.953	5.355	9.308	9.570	102.8
January 21, 1927	a-b-ab	2.377	3.118	5.495	5.468	99.4
	b-a-ab	1.944	1.839	3.783	3.524	92.9
January 30, 1927	b-a-ab	5.313	4.281	9.594	8.940	93.1
February 6, 1927	b-a-ab	5.025	7.193	12.218	10.130	82.9
	b-a-ab	1.016	2.280	3.296	2.941	89.2
February 13, 1927	ab-b-a	6.111	8.895	15.006	15.130	100.7
	a-b ab	7.350	10.110	17.460	20.000	114.5
February 20, 1927	ab-b-a	1.050	2.068	3.121	3.250	104.0
February 27, 1927	ab-b-a	2.426	4.769	7.195	6.661	92.5
	ab-b-a	1.553	4.274	5.827	5.553	95.1
March 6, 1927	ab-b-a	4.292	7.091	11.383	11.100	97.5
	ab-b-a	3.896	6.317	10.213	10.410	101.9
	ab-b-a	3.012	4.621	7.633	7.993	104.7
	a-b-ab	2.658	3.243	5.901	4.743	80.3
	ab-a-b	1.347	1.615	2.962	3.518	118.7
March 13, 1927	ab-b-a	2.134	4.173	6.307	6.668	105.7
	a-b-ab	1.908	3.735	5.643	4.629	81.9
	ab-b-a	1.557	3.120	4.677	4.403	94.1
	a-ab-b	1.514	2.442	3.956	3.711	93.8
March 20, 1927	b-ab-a	2.239	8.521	10.760	9.510	88.3
	a-ab-b	2.135	7.772	9.907	8.500	85.8
April 10, 1927	b-ab-a	4.606	8.928	13.534	12.040	88.6
	b-ab-a	3 128	8.931	12.059	10.840	89.8
	ab-b-a	3.863	8.230	12.093	10.070	83.2

TABLE 2—*Concluded*

DATE	ORDER OF STIMULATION	Ra	Rb	Ra + Rb	Rab	ADDITIVITY $\frac{Rab}{Ra + Rb}$
						per cent
April 24, 1927	ab-b-a	4.450	7.231	11.681	11.700	100.1
	ab-b-a	2.814	4.874	7.688	8.281	107.5
	a-b-ab	3.244	5.437	8.681	8.577	98.8
	a-b-ab	2.888	4.903	7.791	7.688	98.6
	ab-b-a	2.755	4.770	7.525	7.644	101.3
May 1, 1927	ab-b-a	6.629	8.388	15.017	12.530	83.4
	a-b-ab	6.747	8.388	15.135	14.020	92.6
	ab-b-a	6.458	7.423	13.881	12.100	87.1
Average						96.0

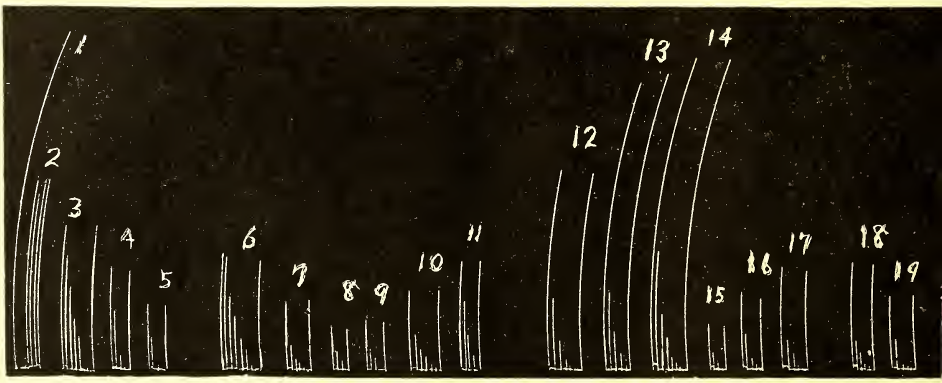

Fig. 4 (reduced to $\frac{2}{3}$ of original)

the screw, S, (fig. 2) of the bone clamp while, by adjusting the screw, V, by means of the handle on the disc, H, (fig. 2) of the dynamometer, the extension indicator, N, was kept at its stop and the stylus at zero. It is thus evident that the lever was after loaded. There is therefore a slight, constant tension on the muscle. This tension, in some cases, was measured by means of weights hung over the pulleys and found to vary from 50 to 80 grams. An interval of 20 seconds was allowed between each successive contraction in determining the maximal tension of the fractions or of the total muscle, with a rest of 3 minutes between experiments. The lengths of the muscles varied in different animals from about 9 to 11 cm. The order of stimulation is indicated in the second column of table 2.

The graphic record of a typical experiment is shown in figure 4. The measurements in grams of this experiment are given in table 2 under the date of April 24, 1927. In figure 4, the first line, *1*, indicates the maximum contraction of the muscle, with a long spring, which is the first step in selecting the proper spring. The first group, *2*, of four marks was made in determining the L.M.C.S. The second group, *3*, of lines was made in determining the maximal tension of the muscle when both nerves were simultaneously excited. The first mark in this group indicates the height of contraction with the initial load. Seven other contractions successively decreasing with increasing loads were recorded before the end point and the maximal tension were reached. The last line in this group is the record of a test contraction made again with the slight initial load for comparison with the first, in order to see how well the reactivity of the preparation had maintained itself through the series of contractions necessary for determining the maximal load. These two contractions taken before and after a determination of maximal tension usually did not differ much. The test contraction was sometimes higher than the first. When it was materially lower than the first the preparation was usually a poor one. These contractions were obtained under conditions of equal length of muscle. The actual tension in the opposing spring in equilibrium with that in the muscles may vary slightly. Usually, however, the change in the zero condition before and after, which is a measure of the change in the tension was not more than 2° or about 0.24 mm. Similarly, group *4* refers to the maximum tension of the muscle when one nerve branch, *a*, is stimulated and group *5* when the other, *b*, is stimulated. Determinations of maximum tension during these contractions yielded additivity of 100.1 per cent. Groups *6*, *7* and *8* yielded 107.5 per cent with a slightly shorter spring; *9*, *10* and *11*, yielded 98.8 per cent. After some adjustments of the electrodes were made, groups *12*, *13* and *14* were recorded with a spring eighteen times as long as the first one (to observe the influence of using a much longer spring), yielding 84.8 per cent. Groups *15*, *16*, *17* and *18*, *19*, *20* were obtained again using the short spring and yielded 98.6 per cent and 101.3 per cent, respectively.

In table 2 are summarized the results of forty experiments performed with the apparatus and technique described. The maximum tension, R*a* in kilograms, measured when the muscle was stimulated through one nerve branch, *a*, is shown in the third column, the tension R*b* for the other nerve branch, *b*, and R*ab* for both branches stimulated simultaneously are shown in the fourth and sixth columns respectively; the arithmetical sum of R*a* and R*b*, in the fifth column. The ratio of R*ab* to R*a* + R*b*, denoted as percentage of additivity, is shown in the seventh column. The average of the forty measurements shown in table 2 is 96.0 per cent. The mean variation is 7.48 per cent which may be compared with the mean variation of 5.5 per cent in the control experiment with both muscles shown in table 1.

Discussion. Several factors suggest themselves as sources of error: 1, the stretch of the muscle and tendon during contraction; 2, asynchronism in the contraction of the two portions of the muscle when simultaneously excited; 3, the obliquity of the muscle fibers; 4, the curve indicating the function relating tension and length of the muscle.

The stretch of the muscle and tendon would affect the results only if the relationship between extension and load were not linear. Over any considerable range of loads one would not expect to find the extensibility constant. In experiments performed upon the muscle-tendon of the lateral head of the gastrocnemius of three decerebrate cats, we found an average extension of 0.6 mm. per kgm. over a series of loads ranging from 1.4 kgm. to 10 kgm. Quednau obtained a figure of 0.5 mm. per kgm. for the tendo achillis of the frog. We found in the additivity experiments, that the last increments of load in determining the maximal tension of the muscle may be in the neighborhood of 0.1 kgm. (occasionally more) for about 0.5 mm. on the drum. These increments are made when the total tension has already reached a magnitude of 4 kgm. to 12 kgm. It is quite possible that in this case the extensibility of the muscle-tendon is not linear and may, therefore, affect results. It is certain that the extensibility of the muscle-tendon does allow muscle fibers to contract without registering on the drum, since portions of the muscle may be seen to contract without movement of the lever.

It is possible to obtain considerable differences in the time of contraction of the two portions of the muscle when they are excited simultaneously through the two nerves, *a* and *b*. In a few experiments bearing upon this point we were able to obtain a difference of several hundredths of a second. But such differences in time are not sufficient to explain the marked differences in additivity that have been reported by others or obtained at times by ourselves. The total contraction covers an appreciable interval of time and the time during which the muscle is contracting at its maximum, for any given stimulation and for any given load, is relatively a large proportion of the total duration of the contraction. The degree of asynchronism would have to be much larger than that actually found, in order to make an appreciable difference in the final tension developed.

The obliquity of the muscle fibers with reference to the central tendon has been said to militate against additivity. Exact information is lacking which would permit one to estimate the magnitude of such an influence. Besides the obliquity of the fibers with reference to the central tendon, there are also to be considered the factors of change of angle of the fibers during contraction, the shift of the central tendon during fractionate response, and the relative amounts of innervation by the two nerves of the two groups of oblique muscle fibers which are separated by the central tendon.

The nature of the tension length relationship is fundamentally involved

in the question of the cause of variations in additivity. Arithmetical summation is to be expected only in case the relationship between tension and length is linear. If the curve of this function is not linear in the region concerned in these summation experiments, one could not expect 100 per cent additivity. An accurate determination of this relationship for the muscles used in the additivity experiments would perhaps explain the attainment of additivity to 100 per cent in some cases and not in others.

We are greatly indebted to Dr. G. N. Stewart for inspiration and guidance and to Dr. J. M. Rogoff for direction and many helpful suggestions throughout this investigation.

SUMMARY

1. Forty measurements are reported of the tension developed in the lateral head of the gastrocnemius of decerebrate cats when it is excited by each nerve separately and by both simultaneously. The sum of the fractionate tensions divided into the tension of the whole muscle averages 96 per cent.

2. It is concluded that muscular tension is essentially additive under the conditions investigated.

3. These results support the uni-axonal conception of muscle innervation.

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4782

Some Effects of Denervation on Muscular Contraction.

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The effect of denervation on contraction of the gastrocnemius of the frog was observed by recording, simultaneously, the myograms of the intact and denervated muscle. Denervation was accomplished by excising 5 to 8 mm. of sciatic nerve from one leg (7 to 21 days prior to the experiment). Thirty experiments were made on winter frogs *(Rana pipiens)*. A gastrocnemius-sciatic preparation was made from the left (operated) and right (intact) legs. Both preparations were then mounted in such a way that the intact nerve-muscle and its writing lever were directly below the denervated muscle and in alignment with its writing lever. The muscles were stimulated by a maximal tetanus, simultaneously, through their nerves, by means of a Harvard inductorium. Stimulation was either by a switch, operated by hand or, when recurrent automatic stimulation was desired, by means of a switch placed on the kymograph and operated by its rotation. An electromagnetic signal placed in the primary circuit and arranged to write upon the drum in alignment with the two muscle levers, recorded the number and duration of the stimulations.

The myograms of denervated muscle present certain peculiar differences from those of normal muscle. The initial speed of contraction as indicated by the steepness of ascent of the lever, is greater in denervated than in intact muscle. All of the records

* The experiments on frogs were performed in the Zoological laboratory of Oberlin College. Grateful acknowledgment is made to Profs. Buddington and Rogers, who kindly placed materials and laboratory facilities at my disposal. The experiments on cats and further experiments on frogs are being carried on in the H. K. Cushing Laboratory of Experimental Medicine.

agree in this particular. This speed factor should result in a diminished latent period for the denervated muscle. In these experiments, however, the technique was not sufficiently refined to demonstrate this difference. The relaxation phase also takes place more quickly than in the intact muscle. The difference is especially marked at the end of the relaxation phase. There appears also to be a shortening of the intermediate phase, or crest of the myogram, although this fact is less easy to demonstrate than the other two. The total duration of the myogram is, therefore, considerably less in the denervated than in the normal muscle. Staircase phenomenon is marked in the intact muscle and is usually absent or very slight in the denervated muscle.

The experiments permit the suggestion that these changes may be due to altered viscosity of the denervated muscle. J. F. Fulton[1] has proposed a similar explanation on the ground of the diminished tension and the increased area of the myograms showing the staircase effect.

Further investigation of these changes in muscle, on frogs and cats, is in progress. Preliminary observations on cats, in which the left sciatic was sectioned, indicate that the elasticity of the denervated muscle, as measured by the stretch produced by equal increments of load, is less than that of intact muscle subjected to the same experimental conditions.

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[1] Fulton, J. F., "Muscular Contraction and the Reflex Control of Movement," 1926, 252.

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On the Effect Produced on the Polarisation of Nerve by Stimulation.
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